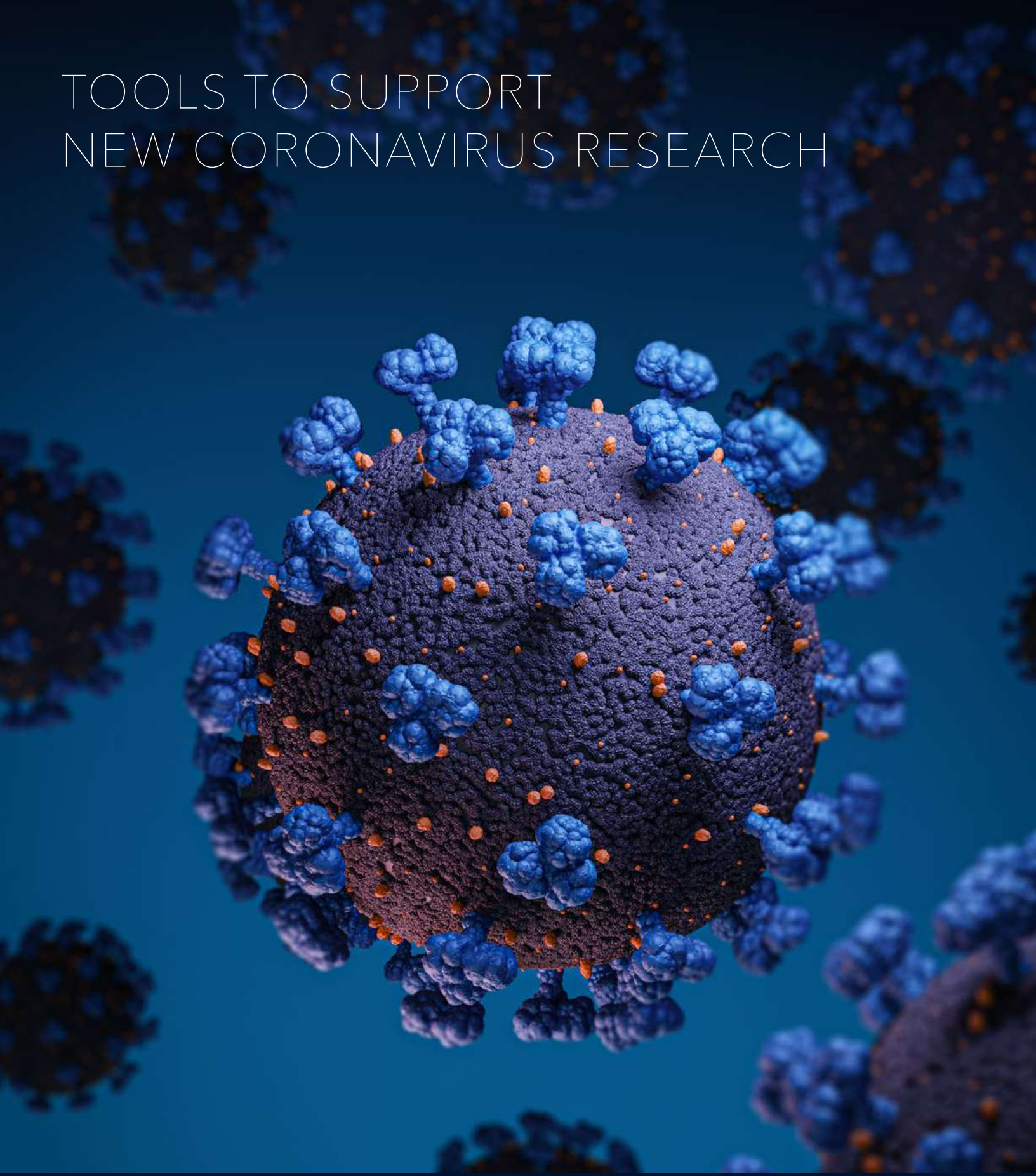
# TOOLS TO SUPPORT NEW CORONAVIRUS RESEARCH



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On February 11, 2020, the new coronavirus (CoV) discovered in Wuhan was officially renamed SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV). It was determined that the virus is genetically related to SARS-like coronaviruses and a strain of the same genus. Coronaviruses are a class of RNA viruses widely found in birds and mammals, including humans. Prior to the emergence of SARS-CoV-2, six coronaviruses had been found to cause human infection. Four strains of coronavirus in circulation globally (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-KHKU1) are responsible for one-third of "common cold" infections. The other two are zoonotic coronaviruses implicated in the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) outbreaks in 2003 and 2012, respectively. Sars-CoV-2 causes a potentially fatal atypical pneurmoia, named Coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). The emergence of this new coronavirus (SARS-CoV-2) will surely be engrained in everyone's memory as the infection continues its course worldwide.

Bio-Techne supports research on the detection and prevention of infectious viruses. As a leading company in the life sciences research sector, Bio-Techne develops resources to support coronavirus research, including tools for SARS-CoV-2 detection, cytokine monitoring, and drug discovery, helping the scientific community understand the mechanisms of infection and develop effective treatments.

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# SARS-COV-2 DIAGNOSIS

Currently, the diagnosis of SARS-CoV-2 infection recommended by the WHO depends on the comprehensive analysis and detection of viral genes, such as the envelope (*E*) and the RNA-dependent RNA polymerase genes, from clinical samples by reverse transcription polymerase chain reaction (RT-PCR). Alternatively, the US Centers for Disease Control and Prevention recommends the detection and analysis of the *N* gene, which encodes a viral nucleocapsid phosphoprotein. Additional diagnostic testing involves serological detection of IgM/IgG antibodies to viral proteins and viral isolation from clinical specimens. Lastly, epidemiological history is part of the clinical evaluation and helps define specific risk factors for infection. Clinical symptoms are mainly fever, dry cough, and shortness of breath, with pneumonia seen in more severe cases. Chest CT scans are used to complement RT-PCR analysis and often reveal pulmonary bilateral ground-glass opacification and infiltrates. Pulmonary consolidation may occur in severe cases. Other indicators evaluated include total number of white blood cells as well as lymph cell and lactate dehydrogenase (LDH) levels. Severe patients often have elevated inflammatory cytokines such as interleukins IL-2, IL-7, and IL-10, granulocyte colony-stimulating factor (G-CSF), and CXCL10/IP-10.

Pulmonary pathological features commonly associated with SARS-CoV-2 include bilateral diffuse alveolar damage, hyaline membrane formation and interstitial mononuclear inflammatory infiltration, as well as unilateral desquamation of pneumocytes and edema. Less frequent findings include multinucleated giant pneumocytes having irregular nuclei distribution, and giant atypical pneumocytes with prominent eosinophilic nucleoli. Flow cytometric analysis of peripheral blood has shown reduced $CD4^+$ and $CD8^+$ T cells that are hyperactivated. In addition, within the $CD4^+$ T cell subset, the frequency of highly proinflammatory CCR4+ and $CCR6^+$ Th17 cells is increased. Severe immune lung damage has been attributed to the increased frequency of proinflammatory Th17 cells and overactive cytotoxic $CD8^+$ T cells.

# SARS-COV-2 TRANSMISSION

SARS-CoV-2 is highly infectious. According to preliminary research, $R_0$ (basic reproduction number) value is 2.2-3.6, which is similar to that of SARS-CoV (3.0) but greater than MERS-CoV (0.8). The fatality rate for SARS-CoV-2 is about 2-3%, which is considerably lower than the fatality rate for both SARS-CoV (~9.5%) and MERS-CoV (~35%) infections. As of March 15, the WHO has reported 153,517 cases of SARS-CoV-2 infections worldwide. Several factors have contributed to its worldwide expansion such as infectiousness level, population movement patterns, and potential asymptomatic transmission. This new SARS-CoV-2 outbreak represents the third instance within the last two decades of the emergence of a highly infectious global threat. SARS-CoV-2 once again has re-focused the public's attention towards the detection and control of infectious viruses.

# SARS-COV-2 DETECTION METHODS

Molecular approaches for the detection of SARS-CoV-2 focus on the analysis of the viral genome and serological analysis of antiviral antibodies. Nucleic acid analysis aims to detect virus-specific genes. Real-time quantitative RT-PCR is the method of choice for SARS-CoV-2 detection because of its high specificity and sensitivity.

Serological analysis detects antibody responses initiated by SARS-CoV-2 infection. Because SARS-CoV-2 is a new human infectious agent, strain specific antibodies are not commonly present in the population. During an immune response, IgM antibodies appear in the early stage, followed by the emergence of IgG during the mid to late disease stages. Recently, an assay that detects IgM and IgG antibodies specific for the nucleocapsid protein from SARS-CoV Rp3 has been developed. In contrast to nucleic acid analysis, serological testing is faster and easier, and offers the advantage of allowing evaluation of convalescent patients. However, a potential key disadvantage to these assays is that antibodies may be undetectable during the early stages of viral infection. Additionally, depending on the antigen used, cross-reactivity, or false positive results, may occur due to the presence of antibodies against closely related CoV strains. These immunological assays are often used to complement nucleic acid analysis for confirming diagnoses of patients with negative nucleic acid findings but clinically suspect. Serological assays are also commonly used for research and surveillance of infectious diseases.

## EXOSOME ANALYSIS WITH EXOSOME DX™

Exosome Diagnostics (ExosomeDx™) is the clinical diagnostic laboratory service of Bio-Techne. It is focused on the development and commercialization of revolutionary biofluid-based diagnostics to deliver personalized precision healthcare that improves lives using exosome analysis. Exosomes are small lipid vesicles that are very similar in structure to enveloped viruses like the coronavirus. ExosomeDx has clinical laboratories for high complexity testing in both the United States (Waltham, MA) and Europe (Munich, Germany). The Waltham CLIA lab is New York state-certified, ISO15189, and has a BSL-2 environment devoted to performing diagnostic testing from a range of different biofluids.

The ExosomeDx platform can yield comprehensive and dynamic molecular insights to transform how diseases are diagnosed, treated, and monitored. To aid with this SARS-CoV2 crisis, we have pivoted resources to help solve the current lack of diagnostic testing. We are available to partner with major health care systems, researchers, and providers to leverage our expertise by developing and performing assays such as:

- SARS-CoV-2 diagnostic qPCR testing
- SARS-CoV-2 serology assays
- Novel RNA biomarkers in plasma to monitor drug treatment and disease-associated responses with complete RNA profiling

The expertise in exosome isolation and biomarker development has made ExosomeDx the "go-to" biomarker platform for many pharmaceutical companies that utilize these capabilities to diagnose, stratify, and monitor patient response in various clinical studies. The proprietary exosome isolation platform enables complete RNA (monitor thousands of targets simultaneously) and protein markers (cell surface and encapsulated) profiling. Exosomes also play a role in normal physiology and monitoring them in biofluids can give information on many different pathways and processes (see the following citations: García-Silva, S. *et al.*, 2019; Hydbring, P. *et al.*, 2018; Krug, A.K. *et al.*, 2018; McKiernan, J. *et al.*, 2018; Simonson, O.E. *et al.*, 2016; Zanello, S.B. *et al.*, 2018). Exosomes have generated significant diagnostic and therapeutic opportunities, including most recently, a clinical trial using therapeutic exosomes for severe COVID-19 (NCT04276987).

Learn more | exosomedx.com

## RNASCOPE™ *IN SITU* HYBRIDIZATION ASSAYS

RNAscope™ assays enable researchers to study SARS-CoV-2 infections in host tissues by chromogenic and fluorescent *in situ* hybridization (ISH). With over 400 publications, researchers worldwide have successfully applied this cutting-edge technology in pursuit of a better understanding of DNA viruses, RNA viruses, and retroviruses. In particular, this molecular virology tool has been used to accurately pinpoint the site and spread of the viral infections in different tissues and organs, as well as in developing and verifying animal models, determining the pathogen-elicited immune response, and for evaluating the efficacy of vaccines. For a multi-omics approach, combining RNAscope™ ISH with immunohistochemistry (IHC) allows spatially resolved gene expression analysis at the RNA and protein level to understand complex multicellular interactions within the tissue.

RNAscope™ uses Advanced Cell Diagnostics' (ACD) unique, patented probe design strategy to enable simultaneous signal amplification and background noise suppression. Unlike traditional ISH methods, its specific double-Z probe design prevents amplification of non-specific signals. In addition, the three double Z probe design and signal amplification increases sensitivity such that even a single molecule of RNA can be detected.

Recently, ACD designed RNAscope™ probes from the SARS-CoV-2 genome. Using the RNAscope™ detection kit and accompanying protocol, SARS-CoV-2 can be detected and visualized in various tissues/organs, similar to studies investigating MERS-CoV (Figure 1). In addition to selecting a predesigned probe for SARS-CoV-2, researchers also have the option to design made-to-order target probes for examining different regions of interest.

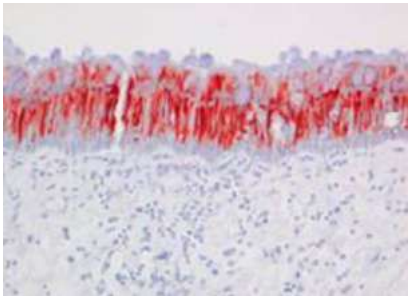


Figure 1. Middle East respiratory syndrome coronavirus (MERS-CoV) is another pathogenic coronavirus. During the development of the viral vaccine, Haagman *et al.* demonstrated the efficacy of the vaccine using RNAscope™ technology. MERS-CoV viral RNA was detected in the nasal respiratory epithelium of camels using an RNAscope probe that specifically targets the nucleocapsid mRNA of the MERS-CoV virus. (positive ISH signal is shown in red). To learn more about this study please refer to Haagmans, B.L. *et al.* (2016) Science **351**:77 (doi: 10.1126/science.aad1283).

*Image Courtesy of Bart L. Haagmans.*

**RNASCOPE FOR SARS-COV-2**

| CATALOG # | PROBE NAME | DESCRIPTION |
|---|---|---|
| 848561 | V-nCoV2019-S | Designed to target the Wuhan-Hu-1 (SARS-CoV-2) complete genome. Optimized to specifically detect the Spike (S) protein of the Novel Coronavirus (COVID-19) to avoid cross detection of SARS-CoV, MERS-CoV, other coronaviruses, Ebola virus or HIV. |
| 845701 | V-nCoV2019-S-sense | A "sense" probe designed to target the antisense strand of the S gene, which can be used to visualize viral replication in individual cells. |
| 848151 | Hs-ACE2 | Provides highly sensitive detection of ACE2 expression in cells and tissue sections. |
| 470341 | Hs-TMPRSS2 | Detects TMPRSS2. |

**SEROLOGY TEST FOR SARS-COV-2**

| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
|---|---|---|---|
| COVID-19 IgG/IgM Rapid Test Kit | Novus Biologicals | NBP2-89106 | Chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies against SARS-CoV-2 in human whole blood, serum, or plasma. |

Learn more | acdbio.com/science/applications/research-areas/infectious-diseases

# THERAPEUTICS RESEARCH AND DEVELOPMENT – DEVELOPING ANTI-COVID-19 DRUGS

Each day the number of confirmed SARS-CoV-2 infections worldwide is rising, creating an urgent need to develop effective treatments. At present, researchers are focused on three key areas for potential treatments: specific antiviral drugs, stem cell therapies, and vaccine development. Drug development for COVID-19 can be classified into four categories:

I. Testing new uses for existing antiviral drugs
II. Screening small molecule libraries
III. Developing antiviral drugs that target specific players in the SARS-CoV-2 life cycle
IV. Monitoring disease progression and targeting the cytokine storm

## TESTING NEW USES FOR EXISTING ANTIVIRAL DRUGS

Developing new drugs that are safe and effective is both time-consuming and costly. However, repurposing existing drugs, also known as drug repositioning, is a strategy that may enable a more rapid response to infectious disease outbreaks. Since the efficacy and safety of FDA approved drugs have been established, the amount of time required to bring a repurposed drug to the market may be dramatically reduced.

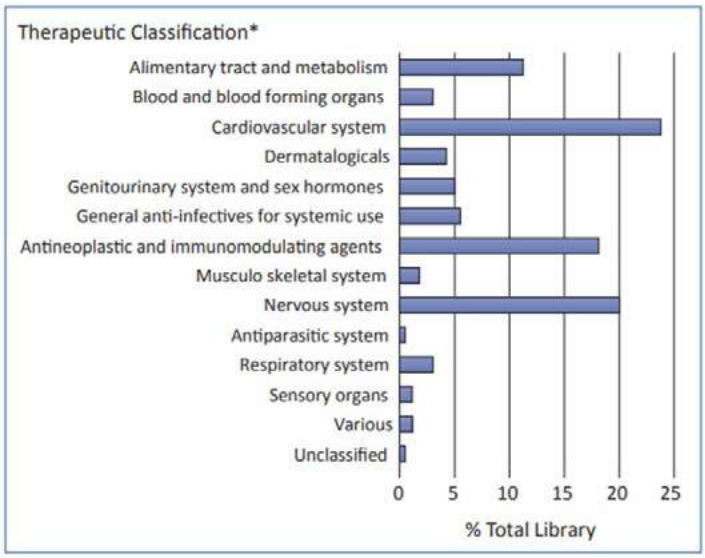



Figure 2. The Tocriscreen Library of FDA-Approved Drugs (Tocris, Catalog # 7200, coming soon) offers 190 FDA-approved compounds supplied pre-dissolved in DMSO. This library of compounds is ideal for screening assays for drug repurposing.

The first step in finding an effective treatment for SARS-CoV-2 is testing existing antiviral drugs developed against SARS, MERS, Ebola, and HIV. For example, the anti-Ebola drug, Remdesivir, which was developed by Gilead Science Inc. and reported positive results after completion of Phase III clinical trials in the United States, is now being investigated as a potential anti-COVID-19 therapeutic by the NIH. In the fight against COVID-19 infection, many clinical studies have also been initiated in China. Kelizhi, marketed as an anti-HIV therapy in the United States, has recently been registered for a clinical trial in combination with interferons in China. Arbidol, which was approved to treat the flu in China, is also being evaluated. Bio-Techne supports the ongoing research to quickly identify a COVID-19 drug by offering a Tocriscreen compound library of 190 FDA-approved drugs that cover over 12 different therapeutic classifications (Figure 2).

## SCREENING SMALL MOLECULE LIBRARIES

Libraries of bioactive compounds can also be screened with the aim of developing a novel therapeutic against COVID-19. Tocris offers a library of 1280 fully annotated compounds for screening and identifying compounds that could target SARS-CoV-2. The Tocriscreen 2.0 compound library, which has very little overlap with other libraries on the market, is available in 3 formats.

| TOCRISCREEN COMPOUND LIBRARIES | | | |
|---|---|---|---|
| | 2.0 MICRO (CATALOG # 7152) | 2.0 MINI (CATALOG # 7151) | 2.0 MAX (CATALOG # 7150) |
| #. of Compounds | 1280 | 1280 | 1280 |
| Volume | 15 µL | 50 µL | 250 µL |
| Solution Format | 10 mM DMSO | 10 mM DMSO | 10 mM DMSO |
| Seal | Peelable foil seal | SepraSeal Cap | SepraSeal Cap |
| Storage Format | 96-well, v-bottom microplate | 96-well racks with Matrix™ storage tubes | 96-well racks with Matrix storage tubes |
| Storage Temperature | -20°C | -20°C | -20°C |
| Stability (for at least) | 6 months, prior to opening | 6 months | 6 months |

Learn more | tocris.com/product-type/tocriscreen-compound-libraries-and-toolboxes

# DEVELOPING ANTIVIRAL DRUGS THAT TARGET SPECIFIC PLAYERS IN THE SARS-COV-2 LIFE CYCLE

SARS-CoV-2 and SARS-CoV are homologous, with their RNA sequences sharing ~80% identity. Research on SARS-host protein interactions with both viruses have shown that the viral spike (S) protein binds human ACE-2 located on the surface of mucosal cells, resulting in fusion of viral and cell membranes for viral entry. Viral entry also requires priming of the S protein by host cell proteases including TMPRSS2 and Cathepsin B/L. Involvement of ADAM17/TACE in this process, which cleaves ACE-2, remains questionable.

Since the S protein, ACE-2, TMPRSS2, and Cathepsin B/L, have important functions in the SARS-CoV-2 life cycle, these proteins serve as potential targets for drug development. A recent study by Hoffmann *et al.* demonstrated that ACE-2 and TMPRSS2 are required for host cell entry. Blocking ACE-2 with an Anti-Human ACE-2 Antibody (R&D Systems, Catalog # AF933) and inhibiting TMPRSS2 with the protease inhibitor Camostat attenuated viral entry. This publication also showed that E 64d (Tocris, Catalog # 4545), a cathepsin inhibitor, prevents viral cell entry *in vitro*, in combination with Camostat. Bio-Techne offers various reagents (Figures 3-10) to support the development of neutralizing antibodies and inhibitors against proteins involved in key steps in SARS-CoV-2 infection.

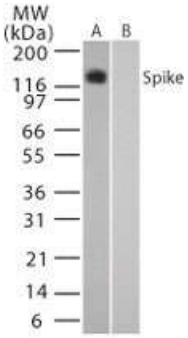



Figure 3. Western blot shows lysates of a mouse melanoma cell line either transfected (**A**) or not transfected (**B**) with a plasmid to express the S protein. The membrane was probed with a Rabbit Anti-SARS Spike Protein Polyclonal Antibody (Novus Biologicals, Catalog # NB100-56578).

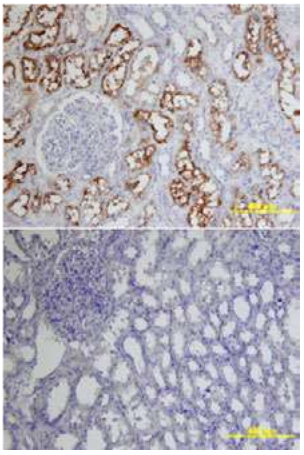


Figure 4. ACE-2 in Human Kidney. ACE-2 was detected in immersion-fixed paraffin-embedded sections of human kidney using a Goat Anti-Human ACE-2 Antigen Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF933). The tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (R&D Systems, Catalog # CTS008; brown) and counterstained with hematoxylin ( blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents

**RECOMBINANT PROTEINS**

| MOLECULE | BRAND | CATALOG # | SPECIES | SOURCE | TAG |
|---|---|---|---|---|---|
| ACE-2 | R&D Systems | 933-ZN | Human | NS0 | No |
| | R&D Systems | 3437-ZN | Mouse | CHO | No |
| Aminopeptidase N/CD13 | R&D Systems | 3815-ZN | Human | NS0 | No |
| | R&D Systems | 2335-ZN | Mouse | NS0 | No |
| Cathepsin B | R&D Systems | 953-CY | Human | NS0 | No |
| Cathepsin B (Native Protein) | Novus Biologicals | NBP1-99197 | Human | Human liver | No |
| Cathepsin B | R&D Systems | 965-CY | Mouse | NS0 | No |
| | Novus Biologicals | NBP2-53084 | Mouse | Baculovirus | No |
| Cathepsin L | R&D Systems | 952-CY | Human | NS0 | No |
| | R&D Systems | 1515-CY | Mouse | NS0 | No |
| CEACAM-1/CD66a | R&D Systems | 2244-CM | Human | NS0 | No |
| DDPIV/CD26 (High Purity Dimer) | R&D Systems | 9168-SE | Human | NS0 | No |
| DDPIV/CD26 | R&D Systems | 954-SE | Mouse | NS0 | No |
| | R&D Systems | 9637-SE | Cynomolgus Monkey | HEK293 | No |
| Furin | R&D Systems | 1503-SE | Human | NS0 | No |
| | R&D Systems | 6450-SE | Mouse | CHO | No |
| Ly6E | R&D Systems | 9970-L6 | Human | HEK293 | No |
| Neutrophil Elastase/ELA2 | R&D Systems | 9167-SE | Human | CHO | No |
| | R&D Systems | 4517-SE | Mouse | NS0 | No |
| SARS-CoV-2 Papain-like Protease | R&D Systems | E-611 | Virus | *E. coli* | No |
| TMPRSS2 | Novus Biologicals | H00007113-Q01 | Human | Wheat germ | No |
| TMPRSS2 Recombinant Protein Antigen | Novus Biologicals | NBP2-38263PEP | Human | *E. coli* | No |
| TMPRSS2 Overexpression Lysate | Novus Biologicals | NBL1-17121 | Human | HEK293T | No |

Learn more | rndsystems.com/proteins

| ANTIBODIES | | | | | | |
|---|---|---|---|---|---|---|
| MOLECULE | BRAND | CATALOG # | SPECIES | CLONE | APPLICATIONS | CONJUGATES AVAILABLE |
| ACE-2 | Novus Biologicals | NBP2-67692* | Human, Mouse, Rat | SN0754 | ICC/IF, IHC, IP, WB | No |
| | Novus Biologicals | NBP2-80035 | Human | AC18F | ELISA, Flow, WB | No |
| | R&D Systems | MAB933 | Human | 171606 | IHC, WB | No |
| | R&D Systems | MAB9331 | Human | 171608 | IP, WB | No |
| | R&D Systems | AF933 | Human | Poly | IHC, IP, SW, WB | Yes |
| | R&D Systems | AF3437 | Mouse | Poly | IHC, IP, SW, WB | No |
| Aminopeptidase N/ CD13 | Novus Biologicals | NBP2-77451 | Human, Mouse, Rat | Poly | Flow, IHC, WB | Yes |
| | Novus Biologicals | NBP2-23492 | Human, Mouse | R3-63 | FA, Flow, ICC/IF, IHC | No |
| | R&D Systems | MAB3815 | Human | 498001 | ICC/IF, IHC, SW, WB | No |
| | R&D Systems | AF3815 | Human | Poly | CyTOF, Flow, IHC, IP, SW, WB | Yes |
| | Novus Biologicals | NB100-64843 | Mouse | ER-BMDM1 | Flow, IHC | Yes |
| | R&D Systems | AF2335 | Mouse | Poly | CyTOF, Flow, ICC/IF, IHC, IP, WB | Yes |
| Cathepsin B | Novus Biologicals | NBP1-19797 | Human, Mouse, Rat | Poly | ICC/IF, IHC, WB | Yes |
| | Novus Biologicals | NBP2-67215* | Human, Mouse | JA11-02 | ICC/IF, IHC, WB | No |
| | R&D Systems | MAB965 | Human, Mouse | 173317 | WB | No |
| | Novus Biologicals | NBP1-86048 | Human | Poly | ICC/IF, IHC | No |
| | R&D Systems | AF953 | Human | Poly | IHC, IP, KO, SW, WB | Yes |
| | Novus Biologicals | NBP1-25931 | Human | Poly | ELISA | FITC |
| | R&D Systems | AF965 | Mouse | Poly | B/N, IHC, WB | Yes |
| Cathepsin L | Novus Biologicals | NB100-1775 | Human, Mouse, Rat + | 33/2 | ELISA, ICC/IF, IHC, MiAR, WB | No |
| | Novus Biologicals | NBP2-67216* | Human, Mouse, Rat | JM10-78 | Flow, ICC/IF, IHC, WB | No |
| | R&D Systems | MAB9521 | Human, Mouse | 204101 | IHC, IP, WB | No |
| | R&D Systems | MAB952 | Human | 204106 | IHC, WB | No |
| | R&D Systems | AF952 | Human | Poly | ELISA, IHC, IP, WB | Yes |
| | R&D Systems | AF1515 | Mouse, Rat | Poly | IHC, SW, WB | Yes |
| CEACAM-1/CD66a | R&D Systems | MAB2244 | Human | 283340 | CyTOF, Flow, WB | Yes |
| | R&D Systems | MAB22441 | Human | 283324 | ELISA, IHC, WB | No |
| | R&D Systems | AF2244 | Human | Poly | IHC, WB | Yes |
| | Novus Biologicals | NBP1-43390 | Mouse | CC1 | B/N, Flow, ICC/IF, IHC, IP, IVT, WB | Yes |
| | R&D Systems | AF6480 | Mouse | Poly | CyTOF, Flow, IHC, WB | No |
| | Novus Biologicals | NBP2-59674 | Rat | 11-1H | ELISA, Flow, ICC/IF, IHC, IP, WB | Yes |
| DDPIV/CD26 | Novus Biologicals | NB100-59021 | Human + | Poly | IHC | No |
| | Novus Biologicals | NBP2-78791 | Human, Mouse, Rat | JM11-42 | ICC/IF, IHC, Flow, WB | No |
| | R&D Systems | MAB1180 | Human | 222113 | CyTOF, ELISA, Flow, WB | Yes |
| | R&D Systems | AF1180 | Human | Poly | ICC/IF, IHC, SW, WB | Yes |
| | R&D Systems | MAB9541 | Mouse | 155202 | CyTOF, Flow | Yes |
| | R&D Systems | AF954 | Mouse | Poly | IHC, SW, WB | Yes |
| | Novus Biologicals | NB100-61658 | Rat | Poly | IHC, PEP-ELISA, WB | No |
| Furin | Novus Biologicals | NB100-1903 | Human, Mouse, Rat + | Poly | B/N, Flow, ICC/IF, IHC, IP, WB | No |
| | Novus Biologicals | NBP2-75495* | Human, Mouse, Rat | JB35-53 | ICC/IF, IHC, WB | No |
| | R&D Systems | MAB15032 | Human | 222712 | ELISA, WB | No |
| | R&D Systems | AF1503 | Human | Poly | IP, WB | Yes |
| | Novus Biologicals | NBP2-26104 | Human | Poly | PEP-ELISA, WB | No |

## ANTIBODIES

| MOLECULE | BRAND | CATALOG # | SPECIES | CLONE | APPLICATIONS | CONJUGATES AVAILABLE |
|---|---|---|---|---|---|---|
| Ly6E | Novus Biologicals | NBP1-52176 | Mouse | Poly | Flow, ICC/IF, PEP-ELISA | No |
| Neutrophil Elastase/ ELA2 | Novus Biologicals | NBP2-61657 | Human, Rat | 6B6G6 | ELISA, Flow, WB | No |
| | Novus Biologicals | NBP2-61658 | Human + | 6B6B10 | ELISA, Flow, IHC, WB | No |
| | R&D Systems | MAB91671R* | Human | 950317R | CyTOF, ICC/IF, IHC, Flow-IC, WB | Yes |
| | R&D Systems | MAB91671 | Human | 950317 | CyTOF, Flow, ICC/IF, IHC, WB | Yes |
| | Novus Biologicals | NBP2-66972* | Human | JF098-6 | Flow, ICC/IF, IHC, WB | No |
| | R&D Systems | MAB4517 | Mouse | 887105 | WB | No |
| SARS Spike Protein | Novus Biologicals | NBP2-24942 | Virus | 17F706 | WB | Yes |
| | Novus Biologicals | NBP2-24746 | Virus | 16F1071 | ICC/IF, WB | Yes |
| | Novus Biologicals | NB100-56578 | Virus | Poly | ICC/IF, WB | No |
| | Novus Biologicals | NB100-56047 | Virus | Poly | ELISA, WB | No |
| | Novus Biologicals | NB100-56048 | Virus | Poly | ELISA, WB | No |
| SARS Nucleocapsid Protein | Novus Biologicals | NB100-56683 | Virus | Poly | WB, ELISA, ICC/IF | No |
| | Novus Biologicals | NB100-56049 | Virus | Poly | WB, ELISA | No |
| | Novus Biologicals | NBP2-24747 | Virus | Ncap11 | WB, ICC/IF | Yes |
| | Novus Biologicals | NBP2-24745 | Virus | 18F629.1 | WB | No |
| SARS Envelope Protein | Novus Biologicals | NB100-56562 | Virus | Poly | WB, ELISA | No |
| SARS Membrane Protein | Novus Biologicals | NB100-56569 | Virus | Poly | WB | No |
| | Novus Biologicals | NBP1-28852 | Virus | 2H2C4 | ELISA, WB | No |
| SARS RDRP | Novus Biologicals | NBP2-50258 | Virus | 4E6 | WB | Yes |
| TMPRSS2 | Novus Biologicals | H00007113-M05 | Human | 2F4 | WB, ELISA | No |
| | Novus Biologicals | NBP1-20984 | Human | Poly | WB, PEP-ELISA | No |
| | Novus Biologicals | H00007113-B01P | Human | Poly | WB | No |

## PEPTIDE SUBSTRATES

| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
|---|---|---|---|
| Mca-YVADAPK(Dnp)-OH Fluorogenic Peptide Substrate | R&D Systems | ES007 | Substrate for ACE-2 and Caspase-1 |
| Z-LR-AMC Fluorogenic Peptide Substrate | R&D Systems | ES008 | Substrate for Cathepsin B, Cathepsin L, and Cathepsin V |

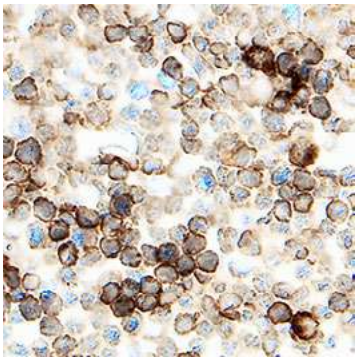


Figure 5. DPPIV/CD26 was detected in immersion fixed frozen sections of mouse thymus using Goat Anti-Mouse DPPIV/ CD26 Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF954). The tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. All cited reagents are from R&D Systems.

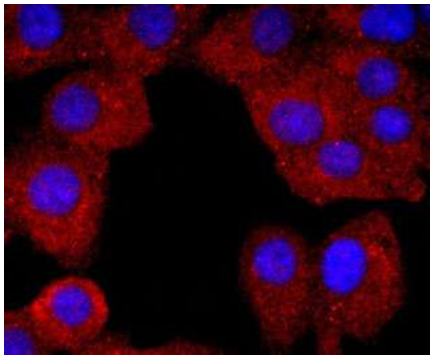


Figure 6. Neutrophil Elastase/ELA2 was detected in fixed A549 human lung carcinoma epithelial cells using a Rabbit Anti-Human Neutrophil Elastase/ELA2 Recombinant Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-66972). Cells were stained red and counterstained with DAPI (blue).

**Species Key: +** Additional Species Available

**Applications Key: B/N** Blocking/Neutralization, **CyTOF** CyTOF-Ready, **ELISA** Capture and/or Detection, **FA** Functional Assay, **Flow** Cytometry, **Flow-IC** Flow Cytometry (Intracellular), **ICC/IF** Immunocytochemistry/Immunofluorescence, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **IVT** *In Vitro*, **KO** Knockout Validated, **MiAR** Microarray, **PEP-ELISA** Peptide ELISA, **SW** Simple Western™, **WB** Western blot

*Indicates a recombinant monoclonal antibody

| SMALL MOLECULES | | | |
|---|---|---|---|
| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
| CHR 2797 | Tocris | 3595 | Aminopeptidase inhibitor, also called Tosedostat |
| MDL 28170 | Tocris | 1146 | Potent and selective Calpain and Cathepsin B inhibitor |
| CA 074 | Tocris | 4863 | Selective Cathepsin B inhibitor |
| Calpeptin | Tocris | 0448 | Calpain and Cathepsin L inhibitor |
| SID 26681509 | Tocris | 3625 | Cathepsin L inhibitor |
| N-Acetyl-L-leucyl-L-leucyl-L-methional | Tocris | 0384 | Cathepsin inhibitor |
| E 64d | Tocris | 4545 | Cathepsin inhibitor; interferes with autolysosomal digestion |
| DPPI 1c | Tocris | 2783 | DPPIV/CD26 inhibitor |
| K 579 | Tocris | 2790 | DPPIV/CD26 inhibitor |
| NVP DPP 728 | Tocris | 3506 | Potent DPPIV/CD26 inhibitor; orally active |
| Saxagliptin | Tocris | 6507 | High affinity DPPIV/CD26 inhibitor; active *in vivo* |
| SSM 3 | Tocris | 5253 | Potent Furin inhibitor |
| ONO 6818 | Tocris | 5651 | High affinity and selective Human Neutrophil Elastase 1 (HNE1) inhibitor; orally active |
| BAY 678 | Tocris | 5706 | Potent Human Neutrophil Elastase (HNE) inhibitor; cell permeable |
| BAY 677 | Tocris | 6389 | Inactive control for BAY 678 (Catalog # 5706) |
| Camostat | Tocris | 3193 | TMPRSS2 inhibitor |

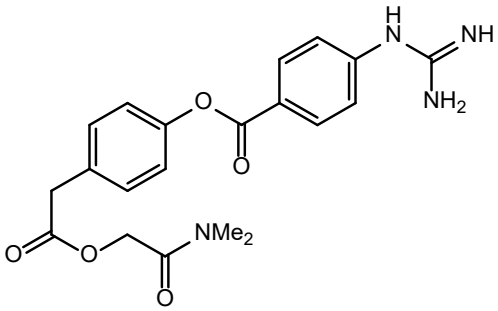



Figure 7. Camostat mesylate (Tocris, Catalog # 3193) is an orally active protease inhibitor. Hoffmann *et al.* showed that camostate mesylate can block SARS-CoV-2 infection of lung cells.

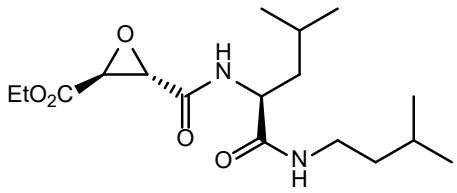



Figure 8. E 64d (Tocris, Catalog # 4545) is an inhibitor of Cathepsin B and L. Hoffmann *et al.* showed that E 64d can inhibit entry of SARS-CoV-2 into cells when combined with the TMPRSS2 inhibitor, Camostat mesylate.

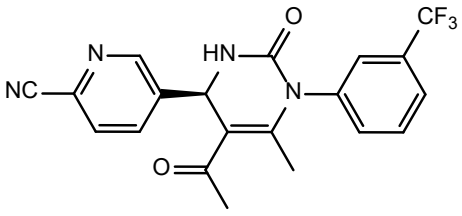



Figure 9. BAY 678 (Tocris, Catalog # 5706) is a potent and selective cell-permeable human Neutrophil Elastase (HNE) inhibitor. Feng *et al.* showed that inhibiting HNE could be a potential therapeutic strategy for treating inflammatory lung diseases such as SARS.

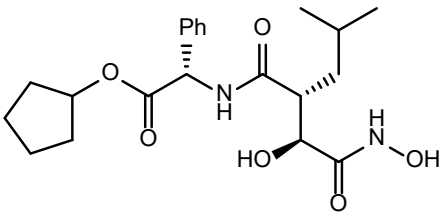



Figure 10. CHR 2797 (Tocris, Catalog # 3595) is an aminopeptidase inhibitor. Yeager *et al.* showed the Aminopeptidase N is a receptor for human coronavirus HCoV-229E.

## MONITORING DISEASE PROGRESSION AND TARGETING THE CYTOKINE STORM

According to reports from the General Office of National Health Commission Office of State TCM Administration: Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 6, Revised), severe and critically ill patients with SARS-CoV-2 often have elevated inflammatory factors. Similarly, a proinflammatory cytokine response has been associated with SARS-CoV infections. Therefore, analysis of inflammatory factors in severe patients may be used to monitor disease progression, disease trends, evaluate prognosis, and guide therapy.

Cytokines (including inflammatory cytokines) are signaling proteins involved in intercellular communication that are released by a broad range of cell types. Within the immune system, cytokines regulate immunological and inflammatory responses. Through the course of viral infections, overactivation of the immune system may lead to excessive production of immune cells and inflammatory cytokines, a process commonly referred to as a cytokine storm or cytokine release syndrome (CRS). SARS-CoV and MERS-CoV infections are frequently associated with inflammatory cellular pulmonary infiltration and elevated proinflammatory cytokines, which lead to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). In severe cases, local lung inflammation may lead to sepsis syndrome. The plasma cytokine profile of patients with pulmonary infection and subsequent sepsis show acute release of tumor necrosis factor- α (TNF-α), IL-β/IL-1F2, IL-8/CXCL8, and CCL2/monocyte chemoattractant protein-1 (MCP-1), followed by sustained release of IL-6. Release of IL-10 follows as the immune system attempts to control the inflammation.

For SARS-CoV-2 infection, recent epidemiological studies have proposed a correlation between elevated levels of inflammatory cytokines and disease severity. Patients infected with SARS-CoV-2 show high levels of IL-1β, IFN-γ, CXCL10/IP-10, and CCL2/MCP-1. Moreover, patients with severe symptoms show significantly higher levels of plasma proinflammatory factors (IL-2, IL-7, IL-10, G-CSF, CXCL10/IP-10, CCL2/MCP-1, CCL3/MIP-1α, TNF-α) than patients with milder symptoms.

These findings suggest that, similar to patients infected with SARS-CoV and MERS-CoV, the levels of cytokine secretion may correlate with the severity of lung lesions in COVID-19 patients. Consequently, therapies that regulate the activity of inflammatory cytokines, such as neutralizing/blocking antibodies or inhibitors, may prove effective at alleviating tissue and organ damage caused by the cytokine storm. Known to suppress cytokine storms in cell therapy, Tocilizumab, a humanized monoclonal antibody against the IL-6 receptor (IL-6 R), has recently been registered in a multicenter, randomized controlled clinical study to evaluate its efficacy for treating COVID-19 (registration number: ChiCTR2000029765).

Cytokine storm monitoring plays a vital role in the evaluation of disease progression. Bio-Techne provides the most comprehensive resources for cytokine analysis and detection, including ELISA kits for individual cytokine detection, Luminex® kits for multiplex cytokine assays, antibody arrays, and automatic ELISA instruments for both quantitative and qualitative detection. Researchers can choose from a large selection of recombinant proteins and antibodies for blocking/neutralization. Biosimilar antibodies, which mimic the effects of existing monoclonal antibodies, and anti-idiotype antibodies, which can be used to evaluate the efficacy of existing antibody drugs, are available to study therapeutic monoclonal antibodies in inflammatory disease. Many of these antibodies are also validated for additional applications including flow cytometry, immunocytochemistry (ICC), IHC, and Western Blot.

### PRODUCTS TO INVESTIGATE THERAPEUTIC MONOCLONAL ANTIBODIES

**BIOSIMILAR ANTIBODIES**

| MOLECULE | BRAND | CATALOG # | SPECIES | CLONE | THERAPEUTIC ANTIBODY | CONJUGATES AVAILABLE |
|---|---|---|---|---|---|---|
| IL-6 R | Novus Biologicals | NBP2-75192 | Human | rhPM-1 | Tocilizumab | No |
| IL-6 R | Novus Biologicals | NBP2-75193 | Human | rhPM-1 | Tocilizumab | No |
| IL-6 Rα | R&D Systems | MAB10346* | Human | Hu137 | Sarilumab | No |
| TNF-α | R&D Systems | MAB9677 | Human | Hu7 | Adalimumab | Yes |
| Integrin α4β7/ LPAM-1 | R&D Systems | MAB10078 | Human | Hu117 | Vedolizumab | Yes |
| CD25/IL-2 Rα | R&D Systems | MAB9926 | Human | Hu107 | Basiliximab | Yes |
| CD25/IL-2 Rα | R&D Systems | MAB9927 | Human | Hu102 | Daclizumab | Yes |

**ANTI-IDIOTYPE ANTIBODIES**

| ANTIBODY | BRAND | CATALOG # | CLONE | CONJUGATES AVAILABLE |
|---|---|---|---|---|
| Adalimumab | R&D Systems | MAB9616* | 2235F | No |
| Adalimumab | R&D Systems | MAB9546 | 972557 | No |
| Daclizumab | R&D Systems | MAB10218* | 2498A | No |

Learn more | proteinsimple.com/products/biosimilar-antibodies

## PRODUCTS TO MONITOR THE CYTOKINE STORM

### SIMPLE PLEX™ ASSAYS

ProteinSimple™ Simple Plex assays are highly sensitive, reproducible immunoassays that will transform your research possibilities. Run on Ella™, these novel assays split samples across isolated microfluidic channels, allowing for the analysis of multiple analytes in very low sample volumes, but with no risk of antibody cross-reactivity. Simple Plex assays exhibit the same specificity of a singleplex ELISA, but with greater sensitivity and a broader dynamic range, providing the highest quality of data in the shortest time to support the rapid detection of antiviral cytokines, evaluate CRS, and study viral immune evasion.

Customize your own multianalyte panel or measure single analytes by choosing from our extensive menu of assays.

| EXAMPLE SIMPLE PLEX CYTOKINE PANELS | |
|---|---|
| PLEX # | ANALYTES DETECTED |
| 8-plex | CCL2/MCP-1, CCL3/MIP-1α, CXCL10/IP-10, IL-1β/IL-1F2, IL-2, IL-7, IL-10, TNF-α |
| 8-plex | IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-6, IL-10, IL-12 p70, TNF-α |
| 4-plex | IL-7, IL-10, IL-12, TNF-α |
| 4-plex* | IL-6, IL-8/CXCL8, TNF-α, IL-1β/IL-1F2 |

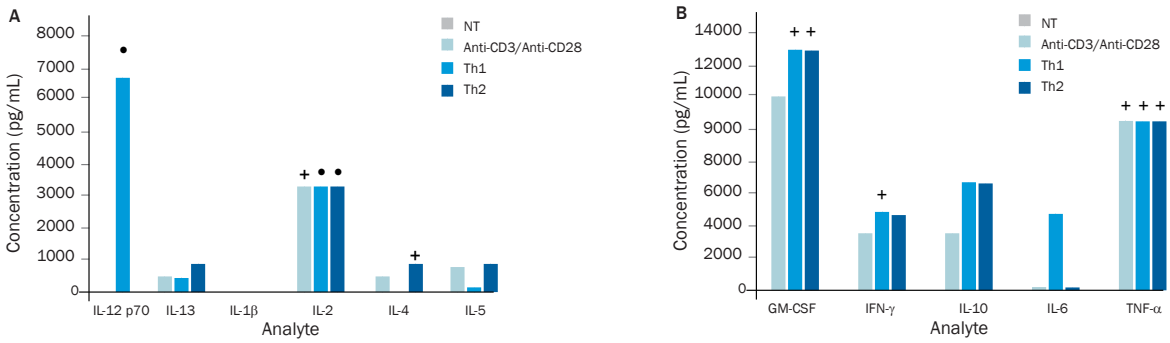
*This panel of analytes has been put together in collaboration with, and on the request of Mt. Sinai Hospital (New York), the Spallanzani Hospital & National Institute for Infectious Diseases in Italy (Rome) for the rapid multicytokine monitoring of COVID-19 patients.

Learn more | proteinsimple.com/ella

### LUMINEX® ASSAYS

Bio-Techne offers two bead-based multiplex immunoassay formats utilizing Luminex xMAP® microparticle technology, allowing users to better tailor assay selection to their individual research needs. Design the multiplexing assay you need for your preliminary investigations with our R&D Systems™ Luminex Assays, which are optimized to simultaneously analyze a wide variety and large number of analytes. Then use our Luminex High Performance panels, which are the most accurate and precise Luminex assays, to customize your own assay to detect antiviral cytokines, evaluate CRS, or detect T cell activation (Figure 11).

| LUMINEX HIGH PERFORMANCE ASSAYS | | |
|---|---|---|
| PRODUCT | MAXIMUM # ANALYTES DETECTED | CATALOG # |
| Human XL Cytokine High Performance Panel | 45 | FCSTM18 |
| CCL2/JE/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL11/Eotaxin, CCL19/MIP-3β, CCL20/MIP-3α, CD40 Ligand/TNFSF5, CX3CL1/Fractalkine, CXCL1/GROα/KC/CINC-1, CXCL2/GROβ/MIP-2/CINC-3, CXCL10/IP-10, FGF basic/FGF2/bFGF, Flt-3 Ligand/FLT3L, G-CSF, GM-CSF, Granzyme B, IFN-α/IFNA2, IFN-β, IFN-γ, IL-1α/IL-1F1, IL-1β/IL-1F2, IL-1ra/IL-1F3, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7 | | |
| Human High Sensitivity Cytokine Panel B | 17 | LBHS000 |
| GM-CSF, IFN-γ, IL-1β/IL-1F2, IL-2, IL-5, IL-6, IL-7, IL-13, IL-15, IL-17A, IL-17F, IL-22, IL-23, IL-31, IL-33, IL-36β, TNF-α | | |
| Human Fixed Th1/Th2 Discovery 11-plex | 11 | LKTM008 |
| GM-CSF, IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p70, IL-13, TNF-α | | |
| NHP XL Cytokine High Performance Panel | 35 | FCSTM21 |
| BDNF, CCL2/MCP-1, CCL5/RANTES, CCL11/Eotaxin, CCL20/MIP-3α, CD40 Ligand/TNFSF5, CXCL2/GROβ, CXCL10/IP-10, CXCL11/I-TAC, CXCL13/BLC, FGF basic/FGF2/bFGF, G-CSF, GM-CSF, Granzyme B, IFN-α, IFN-β, IFN-γ, IL-1β/IL-1F2, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-10, IL-12 p70, IL-13, IL-15, IL-17A, IL-21, PDGF-AA, PDGF-BB, PD-L1, TGF-α, TNF-α | | |




Quantitation of Cytokines in Cell Culture Supernates from Activated Th1 and Th2 Cells. CD4+ T cells were isolated from peripheral blood mononuclear cells (PBMCs) using the MagCellect Human CD4+ T Cell Isolation Kit (R&D Systems, Catalog # MAGH102). Cells were either untreated (NT), treated with Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL), Recombinant Human IL-12 (R&D Systems, Catalog # 219-IL), and Mouse Anti-Human IL-4 Monoclonal Antibody (R&D Systems, Catalog # MAB304) and activated with immobilized Mouse Anti-Human CD3ε Monoclonal Antibody (R&D Systems, Catalog # MAB100) and soluble Mouse Anti-Human CD28 Monoclonal Antibody (R&D Systems, Catalog # MAB342) to induce Th1 differentiation, or treated with Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL) and activated with Phytohemagglutinin-L (PHA) to induce Th2 differentiation. All stimulated cells were then treated with PMA (Tocris, Catalog # 1201; 10 ng/mL) and Ionomycin calcium salt (Tocris, Catalog # 1704; 500 ng/mL) for 24 hours after activation. Cell culture supernates were analyzed using the Magnetic Luminex Performance Human Fixed Th1/Th2 Discovery 11-plex Panel (R&D Systems, Catalog # LKTM008).

+ = Values above the limits of the standard curve • = Stimulating cytokine

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## PROTEOME PROFILER™ ANTIBODY ARRAYS

R&D Systems™ Proteome Profiler Antibody Arrays offer a quick and inexpensive analysis of many analytes simultaneously, in less time than it takes to perform a Western blot (Figure 12-13). Highly cited and rated 5 stars by our customers, these membrane-based arrays are ideal for detecting cytokines released following viral infection.

| PROTEOME PROFILER ANTIBODY ARRAYS | | | |
|---|---|---|---|
| PRODUCTS | DESCRIPTION | CATALOG # | SIZE |
| Human XL Cytokine Array Kit | Contains 4 membranes - each spotted in duplicate with 105 different cytokine antibodies | ARY022B | 1 Kit |
| Adiponectin/Acrp30, Angiogenin, Angiopoietin-1, Angiopoietin-2, Apolipoprotein A1, BAFF/BLyS/TNFSF13B, BDNF, CCL2/JE/MCP-1, CCL3/CCL4 (MIP-1α/MIP-1β), CCL5/RANTES, CCL-7/MCP-3, CCL17/TARC, CCL19/MIP-3β, CCL20/MIP-3α, CD14, CD30 CD40/TNFRSF5, Chitinase 3-like, Complement Component C5/C5a, Complement Factor D, C-Reactive Protein/CRP, Cripto-1, CXCL1/GROα, CXCL4/PF4, CXCL5/ENA-78, CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CXCL12/SDF-1α, Cystatin C, Dkk-1, DPPIV/CD26, EGF, EMMPRIN, Endoglin/CD105, Fas Ligand, FGF basic/FGF2/bFGF, FGF-19, Flt-3 Ligand, G-CSF, GDF-15, GM-CSF, Growth Hormone, HGF, ICAM-1/CD54, IFN-γ, IGFBP-2, IGFBP-3, IL-1α/IL-1F1, IL-1β/IL-1F2, IL-1ra/IL-1F3, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-11, IL-12 p70, IL-13, IL-15, IL-16, IL-17A, IL-18 BPz, IL-19, IL-22, IL-23, IL-24, IL-27, IL-28, IL-31, IL-32 α/β/γ, IL-33, IL-34, Kallikrein 3/PSA, KGF/FGF-7, Leptin, LIF, Lipocalin-2/NGAL, M-CSF, MIF, MMP-9, Myeloperoxidase, Osteopontin (OPN), PDGF-AA, PDGF-AB/BB, Pentraxin 3/TSF-14, RAGE, RBP4, Relaxin-2, Resistin, Serpin E1/PAI-1, SHBG, ST2/IL-1 R4, TFF3, TfR, TGF-α, Thrombopoietin, TIM-1/KIM-1/HAVCR, TNF-α, uPAR, VCAM-1/CD106, VEGF, Vitamin D BP | | | |
| Human Cytokine Array Kit | Contains 4 arrays - each spotted in duplicate with 36 different cytokine antibodies | ARY005B | 1 Kit |
| CCL1/I-309, CCL2/MCP-1, CCL3/CCL4 (MIP-1α/MIP-1β), CCL5/RANTES, CD40 Ligand/TNFSF5, CXCL1/GROα, CXCL8/IL-8, CXCL10/IP-10, CXCL11/I-TAC, CXCL12/SDF-1α, G-CSF, GM-CSF, ICAM-1/CD54, IFN-γ, IL-1α/IL-1F1, IL-1β/IL-1F2, IL-1ra/IL-1F3, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, IL-13, IL-16, IL-17, IL-17E/IL-25, IL-18, IL-21, IL-27, IL-32α, MIF, Serpin E1/PAI-1, TNF-α, TREM-1 | | | |

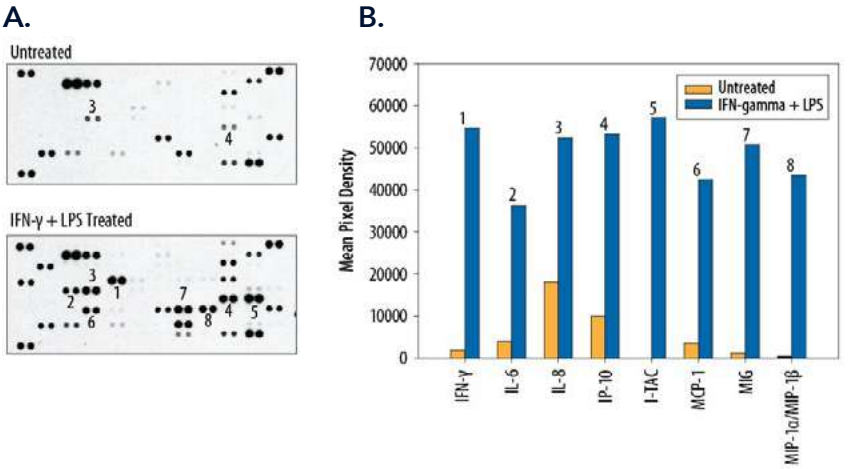



Figure 12. THP-1 human acute monocytic leukemia cells were treated with Recombinant Human IFN-γ (Catalog # 285-IF) for 16 hours, followed by LPS for 8 hours, or remained untreated. Lysates from untreated and treated cells were examined for the levels of 105 different cytokines using the Proteome Profiler Human XL Cytokine Antibody Array (Catalog # ARY022B). Representative arrays (**A**) and histogram profiles (**B**) for select analytes from untreated (orange) and treated (blue) cells. All cited reagents are from R&D Systems.

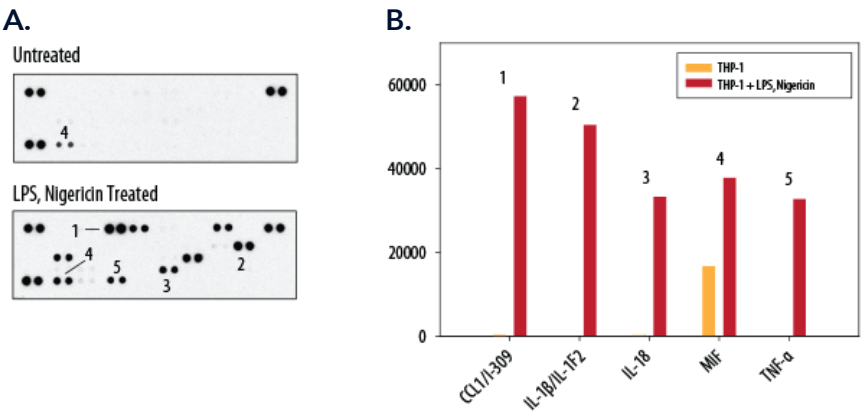



Figure 13. THP-1 human acute monocytic leukemia cells were treated with LPS for 4 hours, followed by the selective K+ ionophore Nigericin (Tocris, Catalog # 4312), or remained untreated. Lysates from untreated and treated cells were examined for the levels of 36 different cytokines using the Proteome Profiler Human Cytokine Antibody Array (R&D Systems, Catalog # ARY005B). Representative arrays (**A**) and histogram profiles (**B**) for select analytes from untreated (orange) and treated (red) cells.

Learn more | rndsystems.com/proteomeprofiler

## ELISAS

Bio-Techne has over 30 years of experience in designing, testing, and optimizing immunoassay kits. Our R&D Systems™ brand ELISAs are the most trusted and most published ELISAs on the market (Figures 14-15). We currently offer kits for 700 target analytes spanning 12 species. R&D Systems Quantikine® ELISA Kits are complete, fully validated, ready-to-run sandwich ELISAs. They are manufactured in-house and undergo extensive validation testing to ensure that they perform as expected every time. R&D Systems™ DuoSet® ELISA Development Systems allow you to develop the immunoassay that you need using our gold-standard ELISA reagents. They are an economical alternative to buying separate antibodies and protein standards when complete kits are not an option.

| QUANTIKINE AND DUOSET ELISAS | | | | |
|---|---|---|---|---|
| ANALYTE | BRAND | SPECIES | QUANTIKINE CATALOG # | DUOSET CATALOG # |
| CCL2/MCP-1 | R&D Systems | Human | DCP00 | DY279 |
| CCL2/JE/MCP-1 | R&D Systems | Mouse | MJE00B | DY479 |
| | R&D Systems | Rat | | DY3144 |
| CCL3/MIP-1α | R&D Systems | Human | DMA00 | DY270 |
| | R&D Systems | Mouse | MMA00 | DY450 |
| CCL4/MIP-1β | R&D Systems | Human | DMB00 | DY271 |
| | R&D Systems | Mouse | MMB00 | DY451 |
| CCL5/RANTES | R&D Systems | Human | DRN00B | DY278 |
| | R&D Systems | Mouse, Rat | MMR00 | DY478 |
| CHI3L1/YKL-40 | R&D Systems | Human | DC3L10 | DY2599 |
| | R&D Systems | Mouse | MC3L10 | DY2649 |
| CX3CL1/Fractalkine | R&D Systems | Human | DCX310 | DY365 |
| | R&D Systems | Mouse | MCX310 | DY472 |
| | R&D Systems | Rat | | DY537 |
| CXCL1/GROα | R&D Systems | Human | DGR00B | DY275 |
| CXCL1/KC | R&D Systems | Mouse | MKC00B | DY453 |
| CXCL1/CINC-1 | R&D Systems | Rat | RCN100 | DY515 |
| CXCL8/IL-8 | R&D Systems | Human* | D8000C | DY208 |
| CXCL10/IP-10 | R&D Systems | Human | DIP100 | DY266 |
| CXCL10/IP-10/CRG-2 | R&D Systems | Mouse | | DY466 |
| GM-CSF | R&D Systems | Human* | DGM00 | DY215 |
| | R&D Systems | Mouse | MGM00 | DY415 |
| | R&D Systems | Rat | | DY518 |
| IFN-α 2/IFNA2 | R&D Systems | Human | | DY9345 |
| IFN-β | R&D Systems | Human | DIFNB0 | DY814 |
| | R&D Systems | Mouse | MIFNB0 | DY8234 |
| IFN-γ | R&D Systems | Human | DIF50 | DY285B |
| | R&D Systems | Mouse | MIF00 | DY485 |
| | R&D Systems | Rat | RIF00 | DY585 |
| IL-1β/IL-1F2 | R&D Systems | Human* | DLB50 | DY201 |
| | R&D Systems | Mouse | MLB00C | DY401 |
| | R&D Systems | Rat | RLB00 | DY501 |
| IL-1ra/IL-1F3 | R&D Systems | Human | DRA00B | DY280 |
| | R&D Systems | Mouse | MRA00 | DY480 |
| IL-2 | R&D Systems | Human* | D2050 | DY202 |
| | R&D Systems | Mouse | M2000 | DY402 |
| | R&D Systems | Rat | R2000 | DY502 |
| IL-4 | R&D Systems | Human* | D4050 | DY204 |
| | R&D Systems | Mouse | M4000B | DY404 |
| | R&D Systems | Rat | R4000 | DY504 |
| IL-6 | R&D Systems | Human* | D6050 | DY206 |
| | R&D Systems | Mouse | M6000B | DY406 |
| | R&D Systems | Rat | R6000B | DY506 |

| QUANTIKINE AND DUOSET ELISAS | | | | |
|---|---|---|---|---|
| ANALYTE | BRAND | SPECIES | QUANTIKINE CATALOG # | DUOSET CATALOG # |
| IL-10 | R&D Systems | Human* | D1000B | DY217B |
| | R&D Systems | Mouse | M1000B | DY417 |
| | R&D Systems | Rat | R1000 | DY522 |
| IL-15 | R&D Systems | Human | D1500 | DY247 |
| | R&D Systems | Mouse | | DY447 |
| IL-17/IL-17A | R&D Systems | Human* | D1700 | DY317 |
| | R&D Systems | Mouse | M1700 | DY421 |
| IL-17A/F Heterodimer | R&D Systems | Human | | DY5194 |
| | R&D Systems | Mouse | M17AF0 | DY5390 |
| IL-18/IL-1F4 | R&D Systems | Human | DL180 | DY318 |
| IL-23 | R&D Systems | Human | D2300B | DY1290 |
| | R&D Systems | Mouse | M2300 | DY1887 |
| TGF-β1 | R&D Systems | Human | DB100B | DY240 |
| | R&D Systems | Mouse Rat + | MB100B | DY1679 |
| TNF-α | R&D Systems | Human* | DTA00D | DY210 |
| | R&D Systems | Mouse* | MTA00B | DY410 |
| | R&D Systems | Rat | RTA00 | DY510 |

*High sensitivity Quantikine ELISAs are available for these analytes.

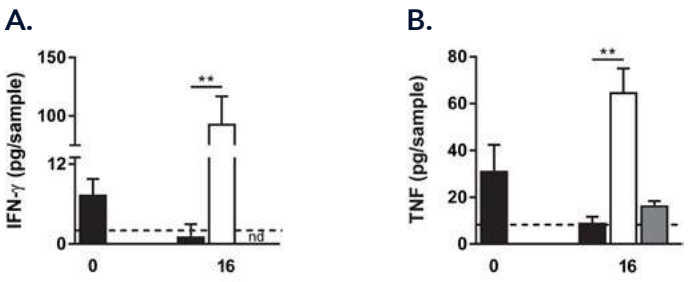



Figure 14. BALB/c mice were infected subcutaneously in the left footpad with *Mycobacterium ulcerans* stain 98-912. After development of a macroscopic lesion, mice were given two doses (s.c. injection) of Lysin B at 10 and 13 days post-infection. Levels of IFN-γ (**A**) and TNF-α (**B**) were quantified in the draining lymph node from non-treated *M. ulcerans* infected mice (black bars), treated *M. ulcerans* infected mice (white bars), and non-infected mice (grey bars) using the R&D Systems Mouse IFN-γ (Catalog # MIF00) and TNF-α (Catalog # MTA00B) Quantikine ELISA Kits. *** p≤0.01. Graph adapted from Fraga, A.G. et al. (2019) PLoS Negl. Trop. Dis.* ***13***:*e0007113.*

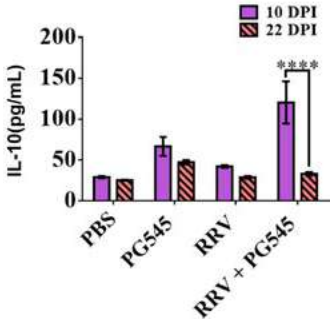



Figure 15. C57BL/6 mice were infected with the Ross River virus (RRV-T48 strain) or injected with PBS as a control. Animals were treated with PG545 (pixatimod), a cholestanol-conjugated, small molecule, heparan sulfate mimetic, or PBS (control) 1 day pre-infection and 4 and 9 days post-infection. Serum levels of IL-10 were analyzed 10 (solid bars) and 22 (stripped bars) days post-infection using the Mouse IL-10 DuoSet ELISA Development System (R&D Systems, Catalog # DY401). *****p<0.0001. Graph adapted from Supramaniam, A. et al. (2019) PLoS ONE* ***14***:*e0217998.*

## ADDITIONAL CYTOKINE-RELATED PRODUCTS

### RECOMBINANT PROTEINS

| MOLECULE | BRAND | CATALOG # | SPECIES | SOURCE | TAG |
|---|---|---|---|---|---|
| CCL2/MCP-1 | R&D Systems | 279-MC | Human | *E. coli* | No |
| | Novus Biologicals | NBP1-81837PEP | Human | *E. coli* | No |
| CCL2/JE/MCP-1 | R&D Systems | 479-JE | Mouse | *E. coli* | No |
| CCL3/MIP-1α | R&D Systems | 270-LD | Human | *E. coli* | No |
| | R&D Systems | 450-MA | Mouse | *E. coli* | No |
| CXCL10/IP-10 | R&D Systems | 266-IP | Human | *E. coli* | No |
| CXCL10/IP-10/CRG-2 | R&D Systems | 466-CR | Mouse | *E. coli* | No |
| G-CSF | R&D Systems | 214-CS | Human | *E. coli* | No |
| | R&D Systems | 414-CS | Mouse | *E. coli* | No |
| IFN-γ | R&D Systems | 285-IF | Human | *E. coli* | No |
| | R&D Systems | 285-GMP | Human | *E. coli* | No |
| | R&D Systems | 485-MI | Mouse | *E. coli* | No |
| IL-1β/IL-1F2 | R&D Systems | 201-LB | Human | *E. coli* | No |
| | R&D Systems | 201-GMP | Human | *E. coli* | No |
| | R&D Systems | 401-ML | Mouse | HEK293T | No |
| IL-4 | R&D Systems | 204-IL | Human | *E. coli* | No |
| | R&D Systems | 204-GMP | Human | *E. coli* | No |
| | R&D Systems | 404-ML | Mouse | *E. coli* | No |
| IL-7 | R&D Systems | 207-IL | Human | *E. coli* | No |
| | R&D Systems | 207-GMP | Human | *E. coli* | No |
| | R&D Systems | 407-ML | Mouse | *E. coli* | No |
| TNF-α | R&D Systems | 210-TA | Human | *E. coli* | No |
| | R&D Systems | 210-GMP | Human | *E. coli* | No |
| | R&D Systems | 410-MT | Mouse | *E. coli* | No |
| VEGF | R&D Systems | 293-VE | Human | *Sf* 21 | No |
| | R&D Systems | 493-MV | Mouse | *Sf* 21 | No |

### ANTIBODIES

| MOLECULE | BRAND | CATALOG # | SPECIES | CLONE | APPLICATIONS | CONJUGATES AVAILABLE |
|---|---|---|---|---|---|---|
| CCL2/MCP-1 | Novus Biologicals | NBP2-22115 | Human, Mouse, Rat + | 2D8 | ELISA, Flow, ICC/IF, IHC, WB | Yes |
| | Novus Biologicals | NBP1-07035 | Human, Mouse, Rat | Poly | B/N, ICC/IF, IHC, WB | Yes |
| | R&D Systems | MAB679 | Human | 23007 | B/N, ELISA, IHC, WB | No |
| | R&D Systems | AF-279-NA | Human | Poly | B/N, IHC, WB | Yes |
| | R&D Systems | AB-479-NA | Mouse | Poly | B/N, ELISA, WB | Yes |
| CCL3/MIP-1α | R&D Systems | MAB670 | Human | 14215 | B/N, ELISA | No |
| | R&D Systems | AF-270-NA | Human | Poly | B/N, ELISA, IHC, WB | Yes |
| | R&D Systems | AF-450-NA | Mouse | Poly | B/N, ELISA, ICC/IF, IHC, WB | Yes |
| CXCL10/IP-10 | R&D Systems | MAB266 | Human | 33036 | B/N, CyTOF, ELISA, Flow | Yes |
| | R&D Systems | AF-266-NA | Human | Poly | B/N, CyTOF, ELISA, Flow, ICC/IF, IHC | Yes |
| CXCL10/IP-10/CRG-2 | R&D Systems | AF-466-NA | Mouse | Poly | B/N, IHC, WB | Yes |

## ANTIBODIES

| MOLECULE | BRAND | CATALOG # | SPECIES | CLONE | APPLICATIONS | CONJUGATES AVAILABLE |
|---|---|---|---|---|---|---|
| G-CSF | R&D Systems | MAB214 | Human | 3316 | B/N, ELISA | No |
| | R&D Systems | AF-214-NA | Human | Poly | B/N, WB | Yes |
| | R&D Systems | MAB414 | Mouse | 67604 | B/N, ELISA, Flow, WB | Yes |
| IFN-γ | R&D Systems | MAB285 | Human | 25718 | B/N, Flow, ICC/IF | Yes |
| | R&D Systems | MAB2852 | Human | K3.53 | B/N, ELISA, Flow, WB | Yes |
| | R&D Systems | MAB485 | Mouse | 37895 | B/N, CyTOF, Flow, WB | Yes |
| IL-1 β/IL-1F2 | Novus Biologicals | NBP1-19775 | Human, Mouse, Rat | Poly | ICC/IF, IHC, WB | Yes |
| | Novus Biologicals | NBP2-27345 | Human | 43N3D8 | Flow, IHC, WB | Yes |
| | R&D Systems | MAB601 | Human | 2805 | B/N, ELISA, ICC/IF, WB | No |
| | R&D Systems | AF-201-NA | Human | Poly | B/N, ICC/IF, IHC, ISH-IHC,WB | Yes |
| | R&D Systems | AF-401-NA | Mouse | Poly | B/N, ICC/IF, IHC, SW, WB | Yes |
| IL-4 | R&D Systems | MAB204 | Human | 34019 | B/N, WB | No |
| | R&D Systems | MAB304 | Human | 3007 | B/N, ICC/IF, WB | Yes |
| | R&D Systems | MAB404 | Mouse | 30340 | B/N, ELISA, WB | No |
| IL-7 | R&D Systems | MAB207 | Human | 7417 | B/N, ELISA, WB | No |
| | R&D Systems | AF-207-NA | Human | Poly | B/N, WB | Yes |
| | R&D Systems | AF407 | Mouse | Poly | B/N, ELISA, WB | Yes |
| TNF-α | Novus Biologicals | NBP1-19532 | Human, Mouse, Rat + | Poly | Flow, ICC/IF, IHC, WB | Yes |
| | R&D Systems | AF-410-NA | Human, Mouse | Poly | B/N, CyTOF, ELISA, Flow, ICC/IF, WB | Yes |
| | R&D Systems | MAB610 | Human | 28401 | B/N, ELISA, ICC/IF, WB | Yes |
| | R&D Systems | AB-410-NA | Mouse | Poly | B/N, WB | No |
| VEGF | R&D Systems | MAB293 | Human + | 26503 | B/N, ELISA, WB | No |
| | R&D Systems | AF-293-NA | Human | Poly | B/N, ICC/IF, IHC, WB | Yes |
| | R&D Systems | AF-493-NA | Mouse | Poly | B/N, ELISA, IHC, WB | Yes |

## SMALL MOLECULES

| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
|---|---|---|---|
| AF 12198 | Tocris | 1793 | Potent, selective human type I IL-1 receptor antagonist |
| CP 424174 | Tocris | 6107 | Inhibitor of IL-1β post-translational processing; indirectly inhibits NLRP3 |
| (D)-(+)-Neopterin | Tocris | 4656 | Stimulated by IFN-γ; marker of immune activation |
| (±)-AMG 487 | Tocris | 4487 | CXCR3 antagonist; inhibits cell migration and metastasis |
| (±)-NBI 74330 | Tocris | 4528 | Potent and selective CXCR3 antagonist |
| VUF 11222 | Tocris | 5668 | High affinity non-peptide CXCR3 agonist |
| C 87 | Tocris | 5484 | TNF-α inhibitor |
| R 7050 | Tocris | 5432 | Inhibitor of TNF-α receptor 1 signaling |
| SKF 86002 | Tocris | 2008 | Inhibits human monocyte IL-1 and TNF-α production; p38 MAP kinase inhibitor |
| GIT 27 | Tocris | 3270 | Immunomodulator; reduces production of pro-inflammatory cytokines |
| JTE 607 | Tocris | 5185 | Cytokine release inhibitor; anti-inflammatory |
| Axitinib | Tocris | 4350 | Potent VEGFR-1, -2 and -3 inhibitor |
| Ki 8751 | Tocris | 2542 | Potent, selective VEGFR-2 inhibitor |

Species Key: + Additional Species Available

**Applications Key: B/N** Blocking/Neutralization, **CyTOF** CyTOF-Ready, **ELISA** Capture and/or Detection, **Flow** Cytometry, I**CC/IF** Immunocytochemistry/Immunofluorescence, **IHC** Immunohistochemistry, **ISH-IHC** Dual ISH-IHC, **SW** Simple Western™, **WB** Western blot

*Indicates a recombinant monoclonal antibody

# PRODUCTS FOR ISOLATING AND IDENTIFYING IMMUNE CELLS

## MAGCELLECT™ CELL SELECTION KITS & REAGENTS

In order to obtain a pure population of immune cells, researchers must often isolate or enrich the cell type of interest from a heterogeneous cell population. R&D Systems™ MagCellect Cell Selection Kits are designed to enrich for specific cell populations based on either a negative or positive selection principle.

**MAGCELLECT CELL SELECTION KITS & REAGENTS**

| PRODUCT | SPECIES | CATALOG # | BRAND |
|---|---|---|---|
| CD14$^+$ Cell Isolation Kit | Human | MAGH105 | R&D Systems |
| CD45$^+$ Cell Isolation Kit | Human | MAGH125 | R&D Systems |
| CD3$^+$ T Cell Isolation Kit | Human | MAGH101 | R&D Systems |
| | Mouse | MAGM201 | R&D Systems |
| | Rat | MAGR301B | R&D Systems |
| Naïve CD4$^+$ T Cell Isolation Kit | Human | MAGH115 | R&D Systems |
| | Mouse | MAGM205 | R&D Systems |
| CD4$^+$ T Cell Isolation Kit | Human | MAGH102 | R&D Systems |
| | Mouse | MAGM202 | R&D Systems |
| | Rat | MAGR302B | R&D Systems |
| Memory CD4$^+$ T Cell Isolation Kit | Human | MAGH116 | R&D Systems |
| | Mouse | MAGM206 | R&D Systems |
| Naïve CD8$^+$ T Cell Isolation Kit | Mouse | MAGM207 | R&D Systems |
| CD8$^+$ T Cell Isolation Kit | Human | MAGH112 | R&D Systems |
| | Mouse | MAGM203 | R&D Systems |
| Natural Killer Cell Isolation Kit | Human | MAGH109 | R&D Systems |
| | Mouse | MAGM210 | R&D Systems |
| MagCellect Magnet | N/A | MAG997 | R&D Systems |
| MagCellect Streptavidin Ferrofluid | N/A | MAG999B | R&D Systems |

## CELLXVIVO™ KITS FOR IMMUNE CELL DIFFERENTIATION OR EXPANSION

Bio-Techne's CellXVivo Immune Cell Differentiation or Expansion Kits contain optimized combinations of the highest quality proteins and antibodies, along with simple, reproducible protocols for the differentiation or expansion of a variety of immune cell types including dendritic cells, macrophages, T helper cell subtypes, and natural killer cells.

**CELLXVIVO KITS**

| PRODUCT | SPECIES | CATALOG # | BRAND |
|---|---|---|---|
| Dendritic Cell Differentiation Kit | Mouse | CDK008 | R&D Systems |
| M1 Macrophage Differentiation Kit | Human | CDK012 | R&D Systems |
| M2 Macrophage Differentiation Kit | Human | CDK013 | R&D Systems |
| Monocyte-derived DC Differentiation Kit | Human | CDK004 | R&D Systems |
| Natural Killer Cell Expansion Kit | Human | CDK015 | R&D Systems |
| Th1 Cell Differentiation Kit | Human | CDK001 | R&D Systems |
| | Mouse | CDK018 | R&D Systems |
| Th17 Cell Differentiation Kit | Mouse | CDK017 | R&D Systems |

## CLOUDZ™ CELL EXPANSION KITS

Bio-Techne's Cloudz Cell Expansion Kits utilize our pioneering Cloudz dissolvable hydrogel for the activation and expansion of specific immune cell populations. Using these dissolvable microspheres eliminates the need to use magnetic particles.

| CLOUDZ KITS | | | |
|---|---|---|---|
| PRODUCT | SPECIES | CATALOG # | BRAND |
| Natural Killer Cell Expansion Kit | Human | CLD004 | R&D Systems |
| Treg Expansion Kit | Human | CLD006 | R&D Systems |

## ANTIBODIES FOR IMMUNE CELL IDENTIFICATION

Flow cytometry is widely used to identify and characterize different immune cell types in heterogeneous samples. It primarily relies on the use of fluorochrome-conjugated antibodies to detect the expression of specific cell surface or intracellular antigens on single cells in suspension. Bio-Techne offers an unparalleled selection of fluorochrome-conjugated R&D Systems and Novus Biologicals antibodies qualified for flow cytometry (Figures 16-18). Hundreds of world-renowned unique clones are available from the R&D Systems brand, many of which have been used to establish CD nomenclature through HLDA workshops. Additionally, the Novus Biologicals brand includes an expansive collection of both proprietary antibodies and some of the most highly referenced antibody clones on the market.

| FLOW CYTOMETRY-VALIDATED ANTIBODIES | | | | |
|---|---|---|---|---|
| CELL SURFACE MOLECULES | SPECIES | CLONE | BRAND | FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME) |
| NAÏVE T CELLS | | | | |
| CD3$^+$ | Human | UCHT1 | R&D Systems | FAB100-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 17A2 | R&D Systems | FAB4841-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 145-2C11 | Novus Biologicals | NBP2-52641* |
| CD45RA$^+$ | Human | MEM-56 | Novus Biologicals | NB500-329-A, C, F, G, N, P, R, S, T, U, V |
| CD45RO$^-$ | Human | UCHL-1 | Novus Biologicals | NBP2-33104 |
| CD62L/L-Selectin$^+$ | Human + | FMC46 | Novus Biologicals | NB100-65388 |
| | Human | DREG56 | Novus Biologicals | NBP1-42795 |
| | Human | 4G8 | R&D Systems | FAB9787-G, R, T |
| | Mouse | MEL-14 | Novus Biologicals | NBP2-81083* |
| CCR7$^+$ | Human | 3D12 | Novus Biologicals | NBP1-43332 |
| | Human | 150503 | R&D Systems | FAB197-A, F, G, N, P, R, S, T, U, V |
| | Human | 150503R | R&D Systems | FAB197R-G, N, R, S, T, U, V* |
| | Mouse | 4B12 | R&D Systems | FAB3477-A, G, N, P, R, S, T, U, V |
| TH1 CELLS | | | | |
| CD3$^+$ | Human | UCHT1 | R&D Systems | FAB100-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 17A2 | R&D Systems | FAB4841-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 145-2C11 | Novus Biologicals | NBP2-52641* |
| CD4$^+$ | Human | RPA-T4 | Novus Biologicals | NBP2-25199 |
| | Human | 13B8.2 | Novus Biologicals | NBP2-52670* |
| | Human | 11830 | R&D Systems | FAB3791-A, C, F, G, N, P, R, S, T, V |
| | Mouse | GK1.5 | R&D Systems | FAB554-A, C, F, G, N, P, R, S, T, U, V |
| T-bet/TBX21$^+$ | Human, Mouse + | 4B10 | Novus Biologicals | NBP1-43298 |
| | Human | 525831 | R&D Systems | FAB53851-G, N, R, S, T, U, V * |
| IFN-γ$^+$ | Human | 25723 | R&D Systems | IC285-A, C, F, G, N, P, R, S, T, U, V |
| TNF-α$^+$ (membrane form) | Human, Mouse + | 52B83 | Novus Biologicals | NB600-1422 |
| | Human | 6401 | R&D Systems | FAB210-F, P |
| | Human | 6402 | R&D Systems | IC210-F, P |

| FLOW CYTOMETRY-VALIDATED ANTIBODIES | | | | |
|---|---|---|---|---|
| CELL SURFACE MOLECULES | SPECIES | CLONE | BRAND | FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME) |
| TH17 CELLS | | | | |
| CD3+ | Human | UCHT1 | R&D Systems | FAB100-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 17A2 | R&D Systems | FAB4841-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 145-2C11 | Novus Biologicals | NBP2-52641* |
| CD4+ | Human | RPA-T4 | Novus Biologicals | NBP2-25199 |
| | Human | 13B8.2 | Novus Biologicals | NBP2-52670* |
| | Human | 11830 | R&D Systems | FAB3791-A, C, F, G, N, P, R, S, T, V |
| | Mouse | GK1.5 | R&D Systems | FAB554-A, C, F, G, N, P, R, S, T, U, V |
| CCR4+ | Human | 205410 | R&D Systems | FAB1567-A, C, F, G, N, P, R, S, T, U, V |
| CCR6+ | Human | 53103 | R&D Systems | FAB195-A, C, F, G, N, P, R, S, T, U, V |
| | Human | 53103R | R&D Systems | FAB195R-G, N, R, S, T, U, V |
| | Mouse | 140706 | R&D Systems | FAB590-A, G, N, P, R, S, T, U, V |
| | Mouse | 140706R | R&D Systems | FAB590R-G, N, R, S, T, U, V* |
| CCR10/GPR2- | Human | 314305 | R&D Systems | FAB3478-A, G, N, P, R, S, T, U, V |
| | Human | 314305R | R&D Systems | FAB3478R-G, N, R, S, T, U, V* |
| | Mouse | 248918 | R&D Systems | FAB2815-A, C, G, N, P, R, S, T, U, V |
| | Mouse | 248918R | R&D Systems | FAB2815R-G, N, R, S, T, U, V* |
| RORα/NR1F1 | Human | 784651 | R&D Systems | IC8924-G, N, P, R, S, T, U, V |
| RORγt/RORC2/NRIF3+ | Human, Mouse | 600380 | R&D Systems | IC6006-A, C, P |
| | Human | 1181A | R&D Systems | IC9125-A, G, N, R, S, T, U, V * |
| IL-17/17A+ | Human | 41802 | R&D Systems | IC3171-A, C, G, N, P, R, S, T, U, V |
| | Human | 41809 | R&D Systems | IC317-A, G, N, P |
| CYTOTOXIC T CELLS | | | | |
| CD3+ | Human | UCHT1 | R&D Systems | FAB100-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 17A2 | R&D Systems | FAB4841-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 145-2C11 | Novus Biologicals | NBP2-52641* |
| CD8+ | Human | 37006 | R&D Systems | FAB1509-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | YTS 105.18 | Novus Biologicals | NBP2-52659* |
| | Mouse | 53-6.7 | R&D Systems | FAB116-A, F, G, N, P, R, S, T, U, V |
| IFN-γ+ | Human | 25723 | R&D Systems | IC285-A, C, F, G, N, P, R, S, T, U, V |

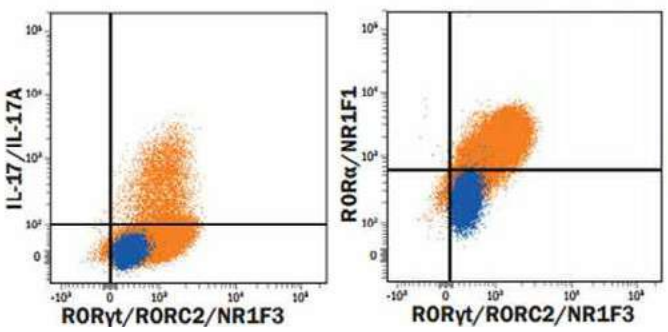



Figure 16. CD4+ T cells were isolated from total human PBMCs using a cell selection protocol, such as the one found in the MagCellect™ Human CD4+ T Cell Isolation Kit (Catalog # MAGH102). Isolated cells were incubated in media containing Recombinant Human IL-2 (Catalog # 202-IL), Human TGF-b1 (Catalog # 100-B), Recombinant Human IL-23 (Catalog # 1290-IL), Recombinant Human IL-6 (Catalog # 206-IL), Recombinant Human IL-1b (Catalog # 201-LB), and a Goat Anti-Human IFN-g Affinity-Purified Polyclonal Antibody (Catalog # AF-285-NA), followed by stimulation with PMA, calcium ionomycin, and monensin. Live, single, CD4+ cells are shown in the dot plots, determined using a fixable viability dye, doublet exclusion, and staining with an Alexa Fluor® 594-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791T). The cells were additionally stained using an Alexa Fluor® 700-conjugated Mouse Anti-Human IL-17/IL-17A Monoclonal Antibody (Catalog # IC3171N), a PE-conjugated Mouse Anti-Human RORa/NR1F1 Monoclonal Antibody (Catalog # IC8924P), and an Alexa Fluor® 488-conjugated Rabbit Anti-Human/Mouse RORgt/RORC2/NR1F3 Monoclonal Antibody (Catalog # IC9125G). Dot plots show the relative IL-17/IL-17A+, RORa/NR1F1+, and RORgt/RORC2/NR1F3+ cells in CD4+ resting (blue dots, lower left) and Th17-differentiated (orange dots, upper right quadrants) cell populations. Quadrant markers were set based on staining with the appropriate isotype controls (Catalog # IC003T, # IC0041N, # IC0041P, and # IC1051G). All cited reagents are from R&D Systems.

| FLOW CYTOMETRY-VALIDATED ANTIBODIES | | | | |
|---|---|---|---|---|
| CELL SURFACE MOLECULES | SPECIES | CLONE | BRAND | FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME) |
| CLASSICAL DENDRITIC CELLS | | | | |
| CD11b/Integrin αM+ | Human, Mouse, Rat + | Poly | Novus Biologicals | NB110-89474 |
| | Human, Mouse + | M1/70.15 | Novus Biologicals | NB600-1327 |
| | Human | ICRF44 | R&D Systems | FAB1699-G, N, R, S, T, U, V |
| | Mouse | M1/70 | R&D Systems | FAB1124-A, F, G, N, P, R, , T, U, V |
| CD11c+ | Human + | BU15 | Novus Biologicals | NBP1-45018 |
| | Human | 3.9 | Novus Biologicals | NB100-2711 |
| | Human | ICRF3.9 | R&D Systems | FAB1777-A, C, G, N, P, R, S, T, U, V |
| | Mouse | N418 | R&D Systems | FAB69501-G, N, R, S, T, U, V |
| HLA-DR+ | Human + | L243 | Novus Biologicals | NB100-77855 |
| | Human | TAL 1B5 | Novus Biologicals | NB600-989 |
| | Human | L203 | R&D Systems | FAB4869-A, C, F, G, N, P, R, S, T, U, V |
| NATURAL KILLER CELLS | | | | |
| CD3- | Human | UCHT1 | R&D Systems | FAB100-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 17A2 | R&D Systems | FAB4841-A, C, F, G, N, P, R, S, T, U, V |
| | Mouse | 145-2C11 | Novus Biologicals | NBP2-52641* |
| CD56/NCAM+ | Human, Mouse, Rat | 735 | Novus Biologicals | NBP2-52669* |
| | Human, Rat + | 123C3 (123C3. D5) | Novus Biologicals | NBP2-33132 |
| | Human | ERIC-1 | Novus Biologicals | NB100-2718 |
| CD127/IL-7Rα- | Human | 40131 | R&D Systems | FAB306-A, G, N, P, R, S, T, U, V |
| | Mouse | 1140A | R&D Systems | FAB7473-G, N, R, S, T, U, V * |
| | Mouse | A7R34 | R&D Systems | FAB47742-A, G, N, P |
| T-bet/TBX21+ | Human, Mouse + | 4B10 | Novus Biologicals | NBP1-43298 |
| | Human | 525831 | R&D Systems | FAB53851-G, N, R, S, T, U, V * |
| EOMES+ | Human | 644730 | R&D Systems | IC6166-A, G, N, R, S, T, U, V |
| | Mouse | 1219A | R&D Systems | IC8889-G, N, P, R, S, T, U, V * |

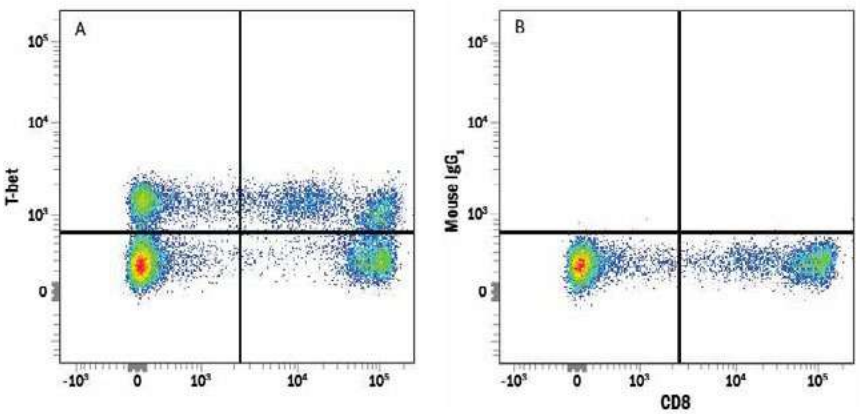



Figure 17. CD4+ Human peripheral blood mononuclear cells (PBMCs) treated with an anti-human IL-4 polyclonal antibody and Recombinant Human IL12 (Catalog # 219-IL) to induce Th1 cell development were stained with a PE-conjugated Mouse Anti-Human IFNg Monoclonal Antibody (Catalog # IC285P) and either an (**A**) Alexa Fluor® 488conjugated Mouse Anti-Human Tbet/TBX21 Monoclonal Antibody (Catalog # IC53851G) or a (**B**) Mouse IgG1 Alexa Fluor 488 Isotype Control (Catalog # IC002G). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). All cited reagents are from R&D Systems.

| FLOW CYTOMETRY-VALIDATED ANTIBODIES | | | | |
|---|---|---|---|---|
| CELL SURFACE MOLECULES | SPECIES | CLONE | BRAND | FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME) |
| M1 MACROPHAGES | | | | |
| B7-1/CD80+ | Human, Mouse | 16-10A1 | Novus Biologicals | NBP1-43385 |
| B7-2/CD86+ | Human, Mouse, Rat | BU63 | Novus Biologicals | NBP2-25208 |
| | Mouse | GL1 | R&D Systems | FAB741-G, N, P, R, S, T, U, V |
| CD68/SR-D1+ | Human, Mouse, Rat + | ED1 | Novus Biologicals | NB600-985 |
| | Human, Mouse, Rat | KP1 | Novus Biologicals | NB100-683 |
| | Human | 298807 | R&D Systems | IC20401-A, F, G, N, P, R, S, T, U, V |
| | Mouse | FA-11 | Novus Biologicals | NBP2-33337 |
| CD163- | Human + | EDHu-1 | Novus Biologicals | NB110-40686 |
| | Human, Rat | GHI/61 | Novus Biologicals | NBP1-43341 |
| | Human | 215927 | R&D Systems | FAB1607-A, C, G, N, R, S, T, U, V |
| | Rat | ED2 | Novus Biologicals | NBP2-39099 |
| HLA-DR+ | Human + | L243 | Novus Biologicals | NB100-77855 |
| | Human | TAL 1B5 | Novus Biologicals | NB600-989 |
| | Human | L203 | R&D Systems | FAB4869-A, C, F, G, N, P, R, S, T, U, V |
| iNOS+ | Human, Mouse | 4E5 | Novus Biologicals | NBP2-22119 |
| M2 MACROPHAGES | | | | |
| CD163- | Human + | EDHu-1 | Novus Biologicals | NB110-40686 |
| | Human, Rat | GHI/61 | Novus Biologicals | NBP1-43341 |
| | Human | 215927 | R&D Systems | FAB1607-A, C, G, N, R, S, T, U, V |
| | Rat | ED2 | Novus Biologicals | NBP2-39099 |
| HLA-DRlow | Human + | L243 | Novus Biologicals | NB100-77855 |
| | Human | TAL 1B5 | Novus Biologicals | NB600-989 |
| | Human | L203 | R&D Systems | FAB4869-A, C, F, G, N, P, R, S, T, U, V |
| MMR/CD206/Mannose Receptor+ | Human | 685641 | R&D Systems | FAB25342-A, G, N, P, R, S, T, U, V |

**Fluorochrome Key: A** Allophycocyanin, **C** PerCP, F Fluorescein, **G** Alexa Fluor® 488, **N** Alexa Fluor 700, **P** Phycoerythrin, **R** Alexa Fluor 647, **S** Alexa Fluor 750, **T** Alexa Fluor 594, **U** Alexa Fluor 350, **V** Alexa Fluor 405. For the full list of available fluorochromes, browse novusbio.com.

*Indicates a recombinant monoclonal antibody

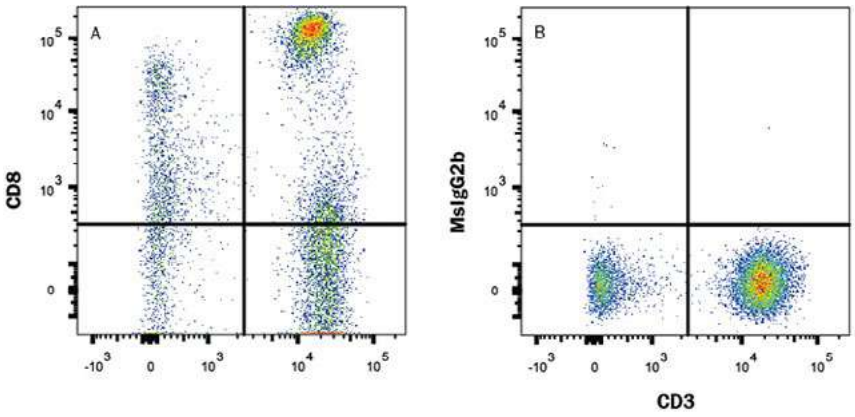



Figure 18. Human peripheral blood lymphocytes were stained with an (**A**) APC-conjugated Mouse Anti-Human CD8a Monoclonal Antibody (Catalog # FAB1509A) or a (**B**) Mouse IgG2B isotype control antibody (Catalog # IC0041A) and a PE-conjugated Mouse Anti-Human CD3e Monoclonal Antibody (Catalog # FAB100P). All cited reagents are from R&D Systems.

# THERAPEUTICS RESEARCH AND DEVELOPMENT – STEM CELL RESEARCH

Mesenchymal Stem Cells (MSCs) are multipotent stem cells with the capacity to differentiate into various cell lineages. MSCs directly participate in the repair of tissue damage and modulate the immune response. MSCs have previously been reported to treat viral infections, specifically, alleviating acute lung injury caused by H5N1 and H9N2 influenza viruses. Production of chemokines and proinflammatory cytokines were reduced and MSCs limited the entry of inflammatory cells into the lungs. Thus, MSCs appear to suppress the adverse effects of the immune response and may relieve symptoms of viral infections by reducing inflammation, inflammatory exudations, and alveolar capillary endothelial cell loss.

While there are no clinically approved stem cell therapies to prevent and treat COVID-19 infections, several research projects have been initiated to investigate the use of MSC as a treatment option. Additionally, using lung organoids to simulate the pulmonary environment can provide a more accurate evaluation of drug efficacy. Bio-Techne offers workflow solutions for MSC and organoid research, including products for isolation, culture, differentiation and identification (Figure 19). We also offer a large supply of GMP cytokines and growth factors for ex vivo cell manufacturing. Full transparency and traceability of source and manufacturing systems is necessary for building a cell therapy product. As such, our large supply of GMP proteins is backed by our dedication to providing cell therapy manufacturers a consistent, safe, and traceable supply of reagents. Browse our full cell and gene therapy manufacturing portfolio at bio-techne.com.

## PRODUCTS FOR MSCS

| MSC ISOLATION KITS, CRYOPRESERVATION MEDIA & CONTAMINATION MONITORING | | | |
|---|---|---|---|
| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
| StemXVivo™ Serum-free MSC Freezing Media | R&D Systems | CCM016 | For serum-free cryopreservation of Human/Mouse/Rat MSCs |
| CryoDefend™-Stem Cells Media | R&D Systems | CCM018 | For defined MSC cryopreservation |
| Y-27632 | Tocris | 1254 | Selective ROCK inhibitor; improves survival rate of stem cells undergoing cryopreservation |
| MagCellect™ Mouse MSC Isolation Kit | R&D Systems | MAGM212B | Uses negative selection to isolate mouse MSCs |
| MycoProbe Mycoplasma Detection Kit | R&D Systems | CUL001B | Detect common antibiotic-resistant cell culture contaminants |

| MSC EXPANSION MEDIA | | | |
|---|---|---|---|
| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
| StemXVivo™ Mesenchymal Stem Cell Expansion Media | R&D Systems | CCM004 | Media for human, mouse, and rat MSCs |
| StemXVivo™ GMP Human MSC Expansion Media | R&D Systems | CCM026 | GMP-grade media for human MSC expansion |
| StemXVivo™ Xeno-Free Human MSC Expansion Media | R&D Systems | CCM021 | Free of non-human animal-derived components |
| StemXVivo™ Serum-Free Human MSC Expansion Media | R&D Systems | CCM014 | Serum free expansion media |

| PROTEINS FOR MSC CULTURING | | | | | |
|---|---|---|---|---|---|
| MOLECULE | BRAND | CATALOG # | GMP VERSION CATALOG # | SPECIES | SOURCE |
| BMP-2 | R&D Systems | 355-BM | 355-GMP* | Human, Mouse, Rat | CHO |
| BMP-4 | R&D Systems | 314-BP | 314-GMP | Human | NS0 |
| EGF | R&D Systems | 236-EG | 236-GMP | Human | *E. coli* |
| FGF basic/FGF2/bFGF (146 aa) | R&D Systems | 233-FB | 233-GMP | Human | *E. coli* |
| IGF-I/IGF-1 | R&D Systems | 291-G1 | 291-GMP | Human | *E. coli* |
| PDGF-BB | R&D Systems | 220-BB | 220-GMP | Human | *E. coli* |
| TGF-β1 | R&D Systems | 240-B | 240-GMP | Human | CHO |
| VEGF 165 | R&D Systems | 293-VE | 293-GMP** | Human | *Sf* 21 |
| Wnt-4 | R&D Systems | 6076-WN | | Human | CHO |
| Wnt-5b | R&D Systems | 7347-WN | | Human | CHO |
| Wnt-10b | R&D Systems | 7196-WN | | Human | CHO |

*The GMP version of BMP-2 is Human only.

**The source for the GMP version of VEGF 165 is *Sf* 9.

| SMALL MOLECULES FOR MSC DIFFERENTIATION | | | |
|---|---|---|---|
| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
| SP 600125 | Tocris | 1496 | Selective JNK Inhibitor |
| SK 216 | Tocris | 6187 | Plasminogen Activator Inhibitor-1 (PAI-1) inhibitor |
| Dexamethasone | Tocris | 1126 | Anti-inflammatory glucocorticoid |
| Zebularine | Tocris | 2293 | DNA methyltransferase and cytidine deaminase inhibitor |
| AICAR | Tocris | 2840 | AMPK activator |
| 5-Azacytidine | Tocris | 3842 | DNA methyltransferase inhibitor |
| TCS 2210 | Tocris | 3877 | Inducer of neuronal differentiation in MSCs |
| Nicotinamide | Tocris | 4106 | PARP-1 inhibitor |
| Kartogenin | Tocris | 4513 | Potently induces chondrogenesis in MSCs |
| Purmorphamine | Tocris | 4551 | Smo receptor agonist |
| Strontium chloride | Tocris | 4749 | Calcium Sensing Receptor (CaSR) agonist |
| GSA 10 | Tocris | 4918 | Smo Receptor agonist |
| Liothyronine sodium | Tocris | 5552 | Thyroid Hormone (T3) analog; also promotes adipogenic differentiation of MSCs |
| KI-7 | Tocris | 6787 | Positive allosteric modulator of A2B receptors |

| MSC IDENTIFICATION KITS, FLOW CYTOMETRY KITS & ANTIBODY PANELS | | | | |
|---|---|---|---|---|
| PRODUCT | SPECIES | BRAND | CATALOG # | DESCRIPTION |
| MSC Functional Identification Kit | Human | R&D Systems | SC006 | Verifies multipotency by *in vitro* functional differentiation |
| | Mouse | R&D Systems | SC010 | |
| | Rat | R&D Systems | SC020 | |
| MSC 4-Color Flow Kit | Human | R&D Systems | FMC002 | Verifies MSC/stromal cell identity by flow cytometry |
| | Mouse | R&D Systems | FMC003 | |
| MSC Verification Flow Kit | Human | R&D Systems | FMC020 | Antibodies for MSC verification according to the markers proposed by the International Society for Cellular Therapy |
| MSC Marker Antibody Panel | Human | R&D Systems | SC017 | Antibody panel for the verification of MSC/stromal cell identity by flow cytometry |
| | Mouse | R&D Systems | SC018 | |

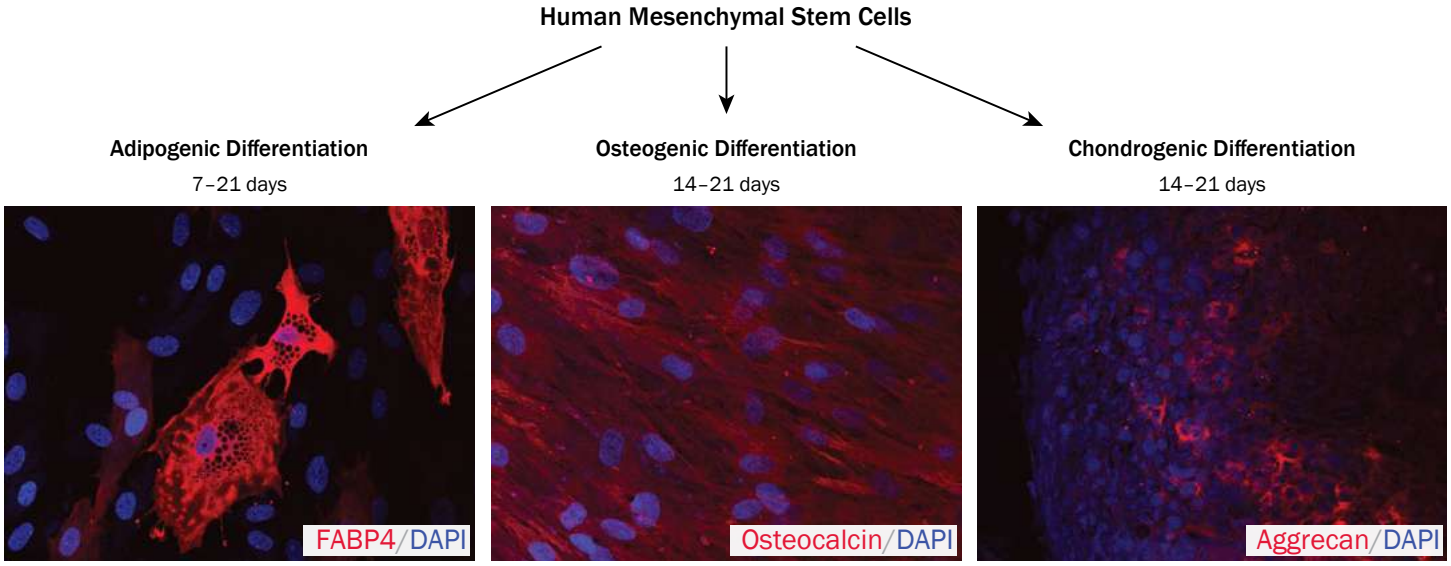



Figure 19. Human mesenchymal stem cells were cultured in StemXVivo™ Mesenchymal Stem Cell Expansion Media (Catalog # CCM004) and differentiation was induced as indicated using the media supplements included in the Human Mesenchymal Stem Cell Functional Identification Kit (Catalog # SC006). The kit also contains a Goat Anti-Mouse FABP-4 Antigen Affinity-Purified Polyclonal Antibody (adipocytes), a Goat Anti-Human Aggrecan Antigen Affinity-Purified Polyclonal Antibody (chondrocytes), and a Mouse Anti-Human Osteocalcin Monoclonal Antibody (Osteocytes) for the confirmation of differentiation status. The cells were stained using the NorthernLights™ 557-conjugated Donkey Anti-Goat (Catalog # NL001; red) or Anti-Mouse (Catalog # NL007; red) IgG Secondary Antibodies, and the nuclei were counterstained with DAPI (blue). All cited reagents are from R&D Systems.

| ANTIBODIES FOR MSC MARKERS | | | | | | |
|---|---|---|---|---|---|---|
| MOLECULE | BRAND | CATALOG # | SPECIES | CLONE | APPLICATIONS | CONJUGATES AVAILABLE |
| 5′-Nucleotidase/ CD73+ | Novus Biologicals | NBP1-85740 | Human, Mouse, Rat + | Poly | ICC/IF, IHC, WB | No |
| | Novus Biologicals | NBP2-48480 | Human | AD2 | Flow, IHC | Yes |
| | R&D Systems | AF5795 | Human | Poly | ICC/IF, IHC, SW, WB | No |
| | R&D Systems | AF4488 | Mouse + | Poly | CyTOF, Flow, ICC/IF, IHC, WB | Yes |
| ALCAM/CD166+ | R&D Systems | AF1172 | Human, Mouse, Rat + | Poly | CyTOF, Flow, ICC/IF, IHC, SW, WB | Yes |
| | Novus Biologicals | NBP1-88129 | Huma, Mouse, Rat | Poly | IHC | No |
| | R&D Systems | MAB6561 | Human | 105902 | CyTOF, ELISA, Flow, WB | Yes |
| | R&D Systems | MAB1172 | Mouse | 200622 | CyTOF, Flow, WB | Yes |
| CD11b- | Novus Biologicals | NB110-89474 | Human, Mouse, Rat + | Poly | Flow, ICC/IF, IHC, ISH, ISH-IHC, SCW, SW, WB | Yes |
| | Novus Biologicals | NB600-1327 | Human, Mouse + | M1/70.15 | CyTOF, Flow, ICC/IF, IHC, IP | Yes |
| CD14- | Novus Biologicals | NBP2-37291 | Human, Mouse | 4B4F12 | CyTOF, ELISA, Flow, ICC/IF, IHC, ISH-IHC, WB | No |
| | Novus Biologicals | NB100-77758 | Human + | M5E2 | B/N, CyTOF, Flow, ICC/IF, IHC | Yes |
| | R&D Systems | MAB3832 | Human | 134620 | B/N, CyTOF, Flow | Yes |
| | R&D Systems | MAB982 | Mouse | 159010 | CyTOF, Flow, WB | Yes |
| CD19- | Novus Biologicals | NBP2-24965 | Human, Mouse, Rat | 1D3 | CyTOF, Flow, IV, IP | Yes |
| | Novus Biologicals | NBP2-25196 | Human, Mouse | CB19 | CyTOF, Flow, ICC/IF, IVT, WB | Yes |
| | R&D Systems | MAB4867 | Human | 4G7-2E3 | CyTOF, Flow | Yes |
| CD34- | Novus Biologicals | NBP2-29455 | Human, Rat | ICO-115 | Flow, ICC/IF, WB | Yes |
| | R&D Systems | AF7227 | Human | Poly | IHC, WB | No |
| | Novus Biologicals | NB600-1071 | Mouse, Rat | MEC 14.7 | ELISA, Flow, ICC/IF, IHC, IP, WB | Yes |
| | R&D Systems | AF4117 | Rat | Poly | IHC, WB | Yes |

Learn more | rndsystems.com/msc

## PRODUCTS FOR LUNG ORGANOIDS

| BASE MEDIA FOR LUNG ORGANOID CULTURE | | | |
|---|---|---|---|
| PRODUCT | BRAND | CATALOG # | DESCRIPTION |
| N-2 MAX Media Supplement (100X) | R&D Systems | AR009 | Fully defined supplement for culturing stem cells; alternative to N-2 |
| GMP N-2 MAX Media Supplement (100X), Animal-free | R&D Systems | AR016 | Serum-free and animal-free media supplement for *ex vivo* cell and tissue manufacturing under GMP-grade culture conditions |
| *N*-Acetylcysteine amide | Tocris | 5619 | Glutathione precursor and cell permeable antioxidant |
| Penicillin-Streptomycin 10/10 (100X) | R&D Systems | B21210 | Contains 10,000 units/mL penicillin and 10,000 g/mL streptomycin |
| Ala-Gln | Tocris | 5823 | Stable form of L-glutamine |
| Organoid Harvesting Solution | R&D Systems | 3700-100-01 | Ready-to-use, non-enzymatic organoid harvesting and dissociation solution |

| 3D GROWTH MATRIX COMPONENTS FOR LUNG ORGANOIDS | | | | | |
|---|---|---|---|---|---|
| PRODUCT/MOLECULE | BRAND | CATALOG # | GMP VERSION CATALOG # | SPECIES | SOURCE |
| Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2, Pathclear | R&D Systems | 3533-010-02 | N/A | N/A | N/A |
| Activin A | R&D Systems | 338-AC | 338-GMP | Human, Mouse, Rat | CHO |
| FGF basic/FGF2/bFGF (146 aa) | R&D Systems | 233-FB | 233-GMP | Human | *E. coli* |
| FGF-4 | R&D Systems | 235-F4 | | Human | *E. coli* |
| Noggin | R&D Systems | 6057-NG | 3344-GMP | Human | NS0 |
| CHIR 99021 | Tocris | 4423 | TB4423-GMP | N/A | N/A |
| SB 431542 | Tocris | 1614 | TB1614-GMP | N/A | N/A |

| ANTIBODIES FOR LUNG ORGANOID MARKERS | | | | | | |
|---|---|---|---|---|---|---|
| MOLECULE | BRAND | CATALOG # | SPECIES | CLONE | APPLICATIONS | CONJUGATES AVAILABLE |
| CKAP4/p63 | Novus Biologicals | NBP1-26642 | Human, Mouse | Poly | WB, IHC, IP | No |
| | R&D Systems | AF7355 | Human | Poly | WB, ICC/IF | No |
| FoxJ1/HFH4 | Novus Biologicals | NBP1-87928 | Human, Mouse | Poly | IHC | No |
| | Novus Biologicals | NBP2-59032 | Human, Mouse | CL3989 | ICC/IF, IHC | No |
| | R&D Systems | MAB3619 | Human | 407003 | CyTOF, Flow | Yes |
| | R&D Systems | AF3619 | Human | Poly | ICC/IF, SW, WB | No |
| HOP | Novus Biologicals | NBP1-97503 | Human, Mouse, Rat + | DS14F5 | IHC, IP, WB | No |
| | Novus Biologicals | NBP1-92003 | Human | Poly | IHC | No |
| ID2 | Novus Biologicals | NBP1-88630 | Human, Mouse, Rat | Poy | ICC/IF, IHC, WB | No |
| | Novus Biologicals | NBP2-66898 | Human, Mouse | A4-D4 | ICC/IF, IHC, WB | No |
| | Novus Biologicals | NBP2-27194 | Human, Rat + | Poly | ICC/IF, WB | No |
| Lgr5/GPR49 | Novus Biologicals | NLS1236 | Human, Mouse, Rat + | Poly | ICC/IF, IHC | No |
| | Novus Biologicals | NBP1-28904 | Human + | Poly | Flow, IHC, WB, ICC/IF (-) | Yes |
| | R&D Systems | MAB8078 | Human | 707042 | CyTOF, FA, Flow, ICC/IF | Yes |
| | R&D Systems | MAB8240 | Mouse | 803420 | CyTOF, FA, Flow, ICC/IF | Yes |
| Prosurfactant Protein C | Novus Biologicals | NBP2-37425 | Human | 5E6A9 | ELISA, WB | No |
| | Novus Biologicals | NBP1-60117 | Human | Poly | IHC, WB | No |
| α-Smooth Muscle Actin | Novus Biologicals | NBP2-33006 | Human, Mouse, Rat + | 1A4/asm-1 | Flow, Flow-IC, ICC/IF, IHC, IP, SW, WB | Yes |
| | Novus Biologicals | NB300-978 | Human, Mouse, Rat + | Poly | ICC/IF, IHC, PEP-ELISA, WB | No |
| TIF-1/NKX2-1 | Novus Biologicals | NBP2-44501 | Human, Mouse, Rat | 8G7G3/1+NX2.1/690 | Flow, ICC/IF, IHC | Yes |
| | Novus Biologicals | NBP2-32999 | Human, Mouse, Rat | SPM150 | Flow, ICC/IF, IHC, IP, WB | Yes |
| | Novus Biologicals | NBP2-41160 | Human, Mouse, Rat | Poly | ELISA, ICC/IF, IHC, WB | No |
| Uteroglobin/SCGB1A1 | Novus Biologicals | NBP2-75705 | Human, Mouse, Rat | JU34-03 | Flow, ICC/IF, WB | No |
| | R&D Systems | MAB4218 | Human | 394324 | IHC, WB | No |
| | R&D Systems | AF4218 | Human | Poly | B/N, IHC | No |

**Species Key: +** Additional Species Available

**Applications Key: B/N** Blocking/Neutralization, **CyTOF** CyTOF-Ready, **ELISA** Capture and/or Detection, **FA** Functional Assay, **Flow** Cytometry, **Flow-IC** Flow Cytometry (Intracellular), **IB** Immunoblotting, **ICC/IF** Immunocytochemistry/Immunofluorescence, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **ISH** *In Situ* Hybridization, **ISH-IHC** Dual ISH-IHC, **IV** *In Vivo*, **IVT** *In Vitro*, **KO** Knockout Validated, **PEP-ELISA** Peptide ELISA, **SCW** Single Cell Western, **SW** Simple Western™, **WB** Western blot

# THERAPEUTICS RESEARCH AND DEVELOPMENT – VACCINE DEVELOPMENT

Development of a SARS-CoV-2 vaccine is the most effective way to prevent future infections. The urgency for such a remedy is driving research into available approaches for vaccine development. Various strategies are being explored including the use of inactivated or attenuated whole virus and structural/functional proteins or peptides as immunogens. One such protein being investigated as a possible immunogen is the S protein, the SARS-CoV-2 protein needed for viral entry. The S protein has been previously shown to induce both neutralizing antibodies and cellular immunity to SARS-CoV in animal models.

From early stage research to vaccine release, Bio-Techne offers innovative and market-leading technologies to support the development and quality control of a COVID-19 vaccine (Figure 20). The pioneering protein analysis solutions from our ProteinSimple brand have already shaped viral-based disease research progress. Our instruments have supported research aimed at developing a new inactivated poliovirus vaccine (Thomassen *et al.*, 2013), detecting norovirus particles particles through capillary isoelectric focusing-whole column imaging detection (CIEF-WCID) (Goodridge *et al.*, 2004), elucidating mechanisms of MERS pathogenesis (Gassen *et al.*, 2019), and developing an identity assay for a 15-valent pneumococcal conjugate vaccine (Hamm *et al.*, 2015). The COVID-19 pandemic presents a dynamic and evolving threat to public health, challenging researchers to quickly understand SARS-CoV-2's pathogenic mechanisms, identify protein targets key to its survival and infectivity, and to develop effective treatments to stop it.

| | SINGLE CELL WESTERN | SIMPLE WESTERN™ | MICRO-FLOW IMAGING | IcIEF AND CE-SDS | SIMPLE PLEX™ |
|---|---|---|---|---|---|
| Cell Line Development | √ | √ | √ | | √ |
| Strain Selection | √ | √ | | | |
| Analytical Development | | √ | √ | √ | √ |
| Cell Bank Development | √ | | | | |
| Upstream Process Development | | √ | √ | | √ |
| GLP Substance Production | | | √ | | |
| Formulation Development | | √ | √ | √ | √ |
| Process Characterization | | | √ | √ | |
| Validation/Documentation | | | | √ | |
| GMP Batch Production | | | | √ | |
| Stability Studies | | √ | √ | √ | |

Figure 20. Bio-Techne's analytical solutions from its ProteinSimple brand that are key for facilitating vaccine development.

## DISCERNING CELL TO CELL VARIABILITY WITH SINGLE CELL WESTERN

A first step in vaccine development is the growth and expansion of cell lines producing candidate viral antigens. Knowing the clonality of the cell line is key and Single-Cell Westerns (scWs) facilitate the detection of cell-to-cell variation. Milo™, Bio-Techne's scW platform, generates robust Western-based protein expression data while measuring target expression heterogeneity and cell type heterogeneity (Figure 21). The fast, automated scW workflow enables analysis of ~1,000 single cells per run, multiplexing up to four proteins per cell, and uses conventional Western blot antibodies.

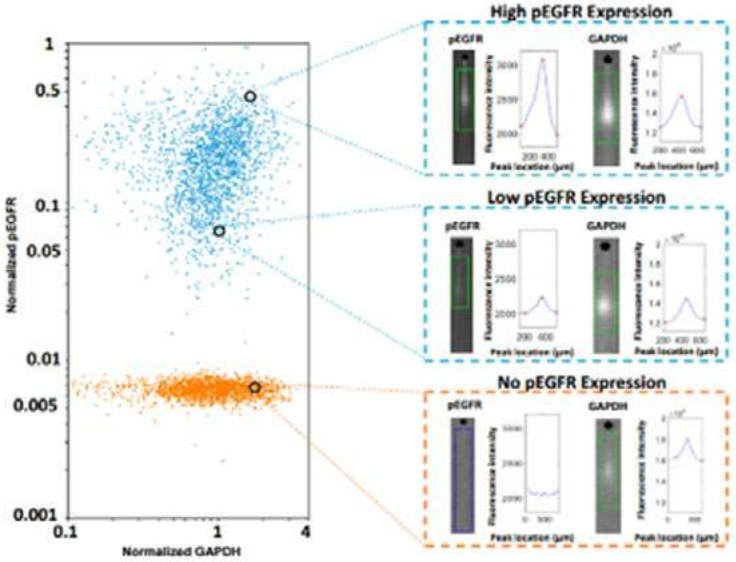



Figure 21. EGFR expression and phosphorylation were analyzed by scW. Positive pEGFR populations (blue) are easily separated from negative pEGFR populations (orange). The cluster of pEGFR positive cells can be further subdivided into high/low pairing.

## ELUCIDATING THE MECHANISM OF VIRAL INFECTIONS BY SIMPLE WESTERN™ ANALYSIS: A MERS-COV CASE STUDY

In studies to uncover the mechanism of MERS infection, Gassen *et al.* (2019) showed that MERS-CoV benefits from reducing autophagy of its host by blocking AP-lysosome fusion. They used Simple Western analysis to show that MERS-CoV infection significantly reduced ATG14 oligomerization (Figure 22), which plays an important role in autophagic activity by promoting autophagosome fusion to the lysosome. They also showed that ATG14 oligomers were enhanced following inhibition of the S-phase Kinase-associated Protein 2 (SKP2) E3 ligase (Figure 23). Quantitative analysis using Simple Western assays showed an approximate 2.5 fold increase in ATG14 oligomers (Figure 23). These results suggest that SKP2 inhibition promotes autophagy and reduces MERS-CoV infection.

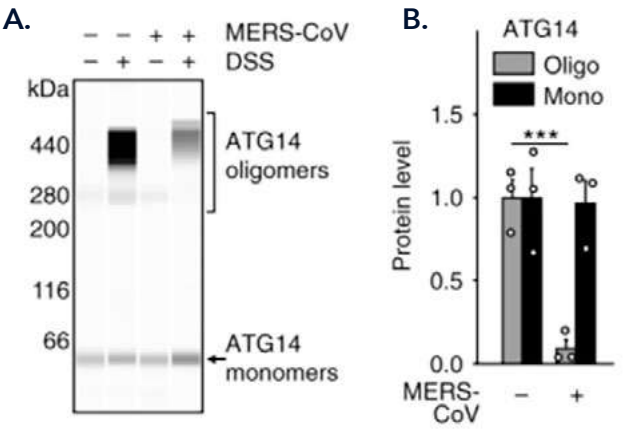



Figure 22. VeroB4 cells were infected with MERS-CoV (MOI= 0.001), cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 minutes and harvested. ATG14 homo-oligomerization was examined with Simple Western (A) and quantified (B). ****p<0.001. Image adapted from Gassen et al. (2019) Nat. Commun.* ***10****:5770.*

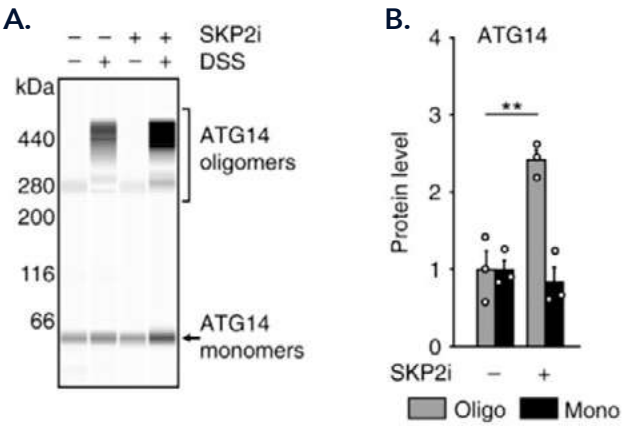
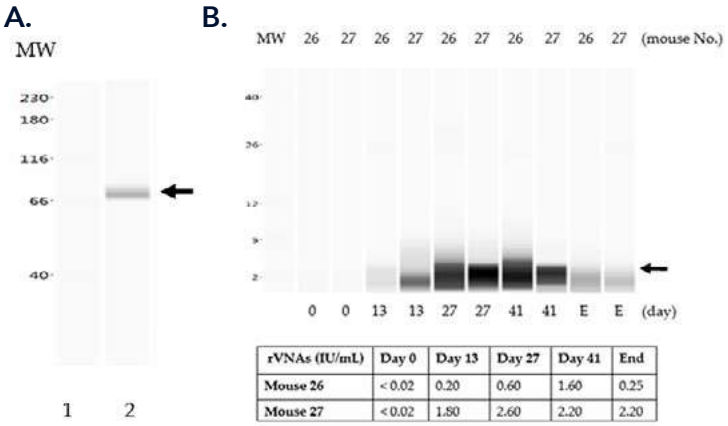



Figure 23. VeroB4 cells were infected with MERS-CoV (MOI= 0.001), treated with SKP2i for 48 h, cross-linked with disuccinimidyl suberate (DSS, 75 µM) 48 h p.i. for 30 minutes and harvested. ATG14 homo-oligomerization was assessed with Simple Western (A) and quantified (B). ***p<0.01. Image adapted from Gassen et al. (2019) Nat. Commun.* ***10****:5770.*

Simple Western is the first fully automated and complete solution for protein detection and characterization, removing the manual, error-prone steps in standard Western blots. In Simple Western assays, proteins can be separated by charge or size from 2 – 440 kDa and detected in as little as 3 hours. Protein is quantified by immunoprobing or total protein labeling with the ability to process up to 96 samples at once. Bio-Techne also offers over 1,300 primary antibodies from its R&D Systems and Novus Biologicals brands that are validated for Simple Western.

## DETECTION AND CHARACTERIZATION OF HOST RESPONSES TO VACCINES WITH SIMPLE WESTERN™ ANALYSIS

Tracking the impact of a vaccine on a recipient is critical for understanding and improving vaccine effectiveness. Wu *et al*. (2019) developed a vaccine against rabies (ERA-2GnRH) that contains a sterilizing agent (GnRH) to control animal populations. They used Simple Western to track the immune response following vaccine administration in mouse models. They observed the rise and fall in antibody levels against GnRH, which corresponded with the rabies viral neutralizing antibodies (rVNAs) (Figure 24). They concluded that there was a concurrent immune response between the GnRH antigen and its vector RABV.




| rVNAs (IU/mL) | Day 0 | Day 13 | Day 27 | Day 41 | End |
|---|---|---|---|---|---|
| Mouse 26 | < 0.02 | 0.20 | 0.60 | 1.60 | 0.25 |
| Mouse 27 | < 0.02 | 1.80 | 2.60 | 2.20 | 2.20 |

Figure 24. Simple Western was used to detect recombinant protein G-2GnRH (A, lane 1) and recombinant G-2GnRH protein from purified ERA-2GnRH virus (A, lane 2). GnRH antibodies were detected in mice at various times points after vaccination (B). *Image adapted from Wu et al. (2019) Vaccines* ***7****:73.*

## CHARACTERIZATION AND DETECTION OF VACCINE IMPURITIES WITH SIMPLE WESTERN™

Bovine serum albumin (BSA), is often an important gauge for quality control in vaccine manufacturing The WHO sets limits on the level of residual impurities allowed in vaccines, including residual BSA. When analyzing BSA levels, it is important to separate the monomeric form from BSA aggregates or degraded products, both of which are indistinguishable from the monomer by ELISA. While SDS-PAGE provides a relatively easy way to measure BSA, Loughney *et al*. (2014) found that a key viral protein antigen for their vaccine migrated at the same molecular weight as BSA. With Simple Western, the Vaccine Analytical Development team at Merck developed a fast and sensitive assay to specifically monitor BSA levels throughout vaccine production and in the final product.

## IMAGED cIEF: A CRITICAL TOOL FOR VACCINE DEVELOPMENT AND MONITORING VACCINE STABILITY

Biopharmaceuticals are often large molecules with complex structures carrying various post-translational modifications (e.g., oxidation, glycosylation, glycation, and deamination) that may lead to changes in their charge distribution. Charge heterogeneity analysis is required for the batch release of biologics as it can be altered by stress, manufacturing changes, age, or other factors. Because charge variants may have different tissue distribution and pharmacokinetics, their characterization is a production quality requirement ensuring consistent biological activity. Imaged capillary isoelectric focusing (icIEF) is the industry gold-standard for charge variant analysis of biopharmaceuticals. Many researchers perform icIEF assays using Bio-Techne's ProteinSimple brand iCE3 or Maurice systems for the development and manufacture of therapeutic monoclonal antibodies, and for characterizing viruses and vaccines.

Merck leveraged the icIEF platforms, iCE3 and Maurice, to study vaccine stability and identity. They used Maurice to analyze lipid nanoparticles (LNPs), which have recently shown great promise as efficient drug delivery systems, by icIEF (Figure 25). In this platform, proteins are measured directly with absorbance or using native fluorescence. The observed that LNPs with different cationic lipids have unique pIs (Figure 25). They also saw that increasing the temperature shifted the pIs to lower values and produced a new peak, which was analogous to acidic variants (Figure 26). Characterization of these charge profiles by cIEF revealed critical differences that may have been missed by size-based separation approaches.

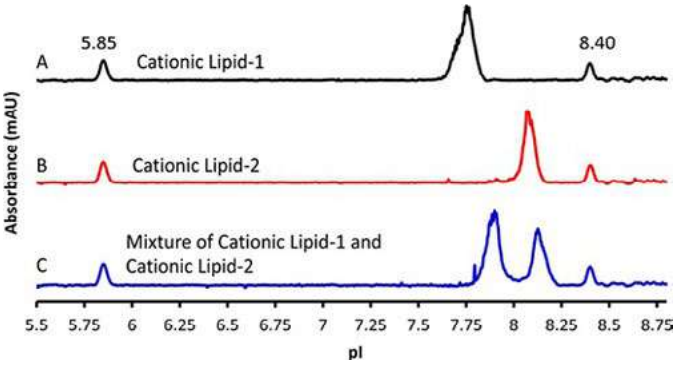



Figure 25. icIEF analysis of LNPs with Bio-Techne's ProteinSimple brand Maurice show different cationic lipids have unique pIs. *Image from Loughney, J.W. et al. (2019) Electrophoresis* ***40****:2602.*

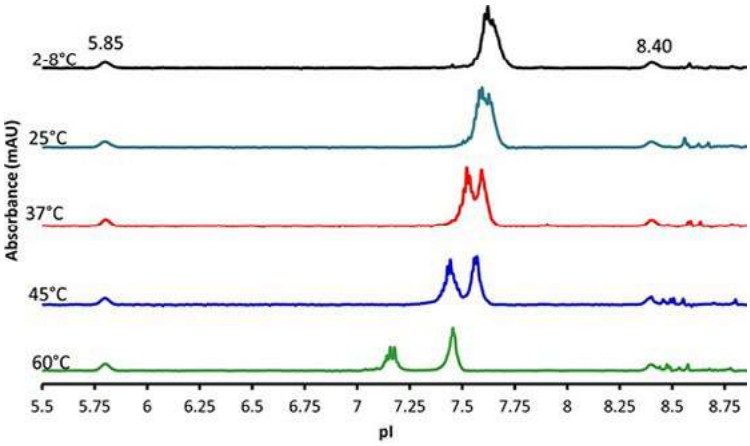



Figure 26. LNPs containing mRNA that were stored under elevated temperatures were analyzed with cIEF. The LNP stored at 2–8°C showed a symmetrical peak shape with a pI of approximately 7.7. As the temperature increased, the LNP with mRNA peaks became more acidic and split into two distinct peaks. *Image from Loughney, J.W. et al. (2019) Electrophoresis* ***40****:2602.*

## CHARACTERIZING VACCINE PARTICULATES WITH MICRO-FLOW IMAGING (MFI)

Sub-visible particulate contaminants are a cause for concern in pharmaceutical products. These contaminants, either from an intrinsic or extrinsic source, may include glass, silicon oil, rubber, and product aggregates. These particulates impact safety and efficacy of products, requiring continuous monitoring throughout production and in the final products. Similar to other biopharmaceuticals, vaccine candidates are often put through forced degradation studies to support analytical method development, obtain information on degradation, and identify optimal conditions and potential stabilizers for long-term storage. Whitaker *et al.* used MFI to assess the aggregation propensities and overall stabilities of trimers of the HIV-1 candidate vaccines, GT1.1 and SOSIP.664 gp140, during agitation (Figure 27). They found these parameters to be comparable when both trimers were formulated in the same buffer.

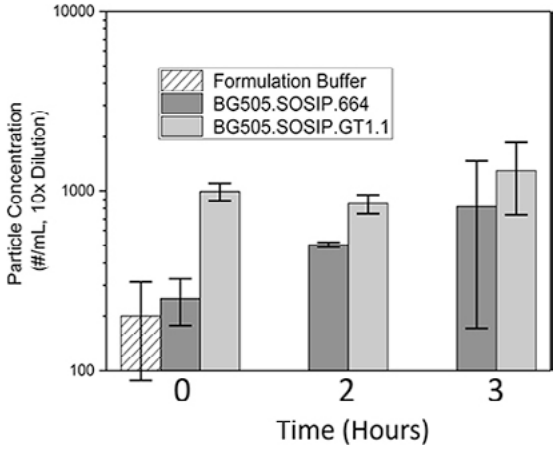



Figure 27. Subvisible (2-100 mm) particle formation (# of particle/mL) during agitation stress studies of BG505 SOSIP.664 and GT1.1 gp140 trimers as determined by MFI. *Graph from Whitaker, N. et al. (2019) J. Pharm. Sci.* ***108****:2264.*

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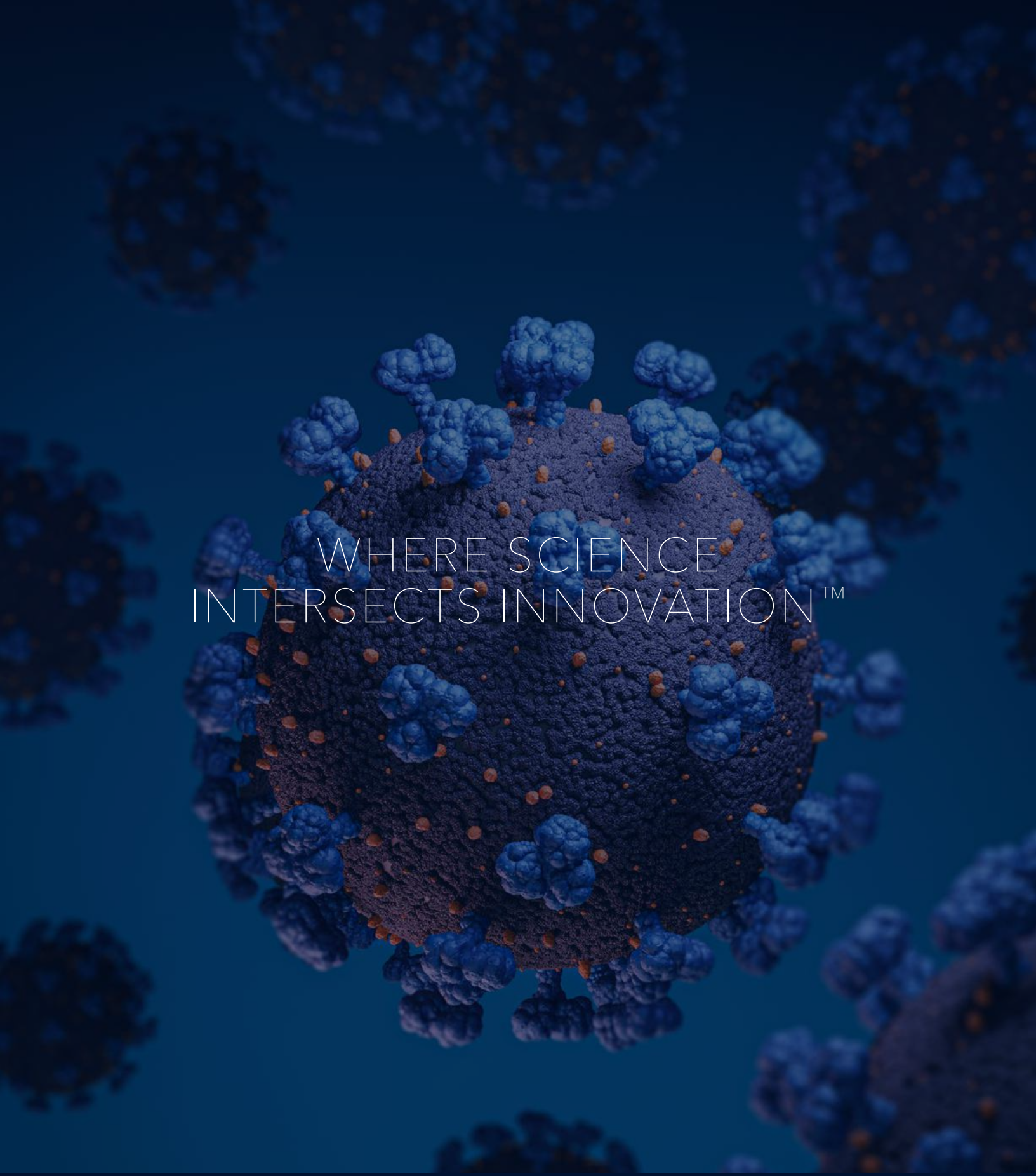
# NOTES

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