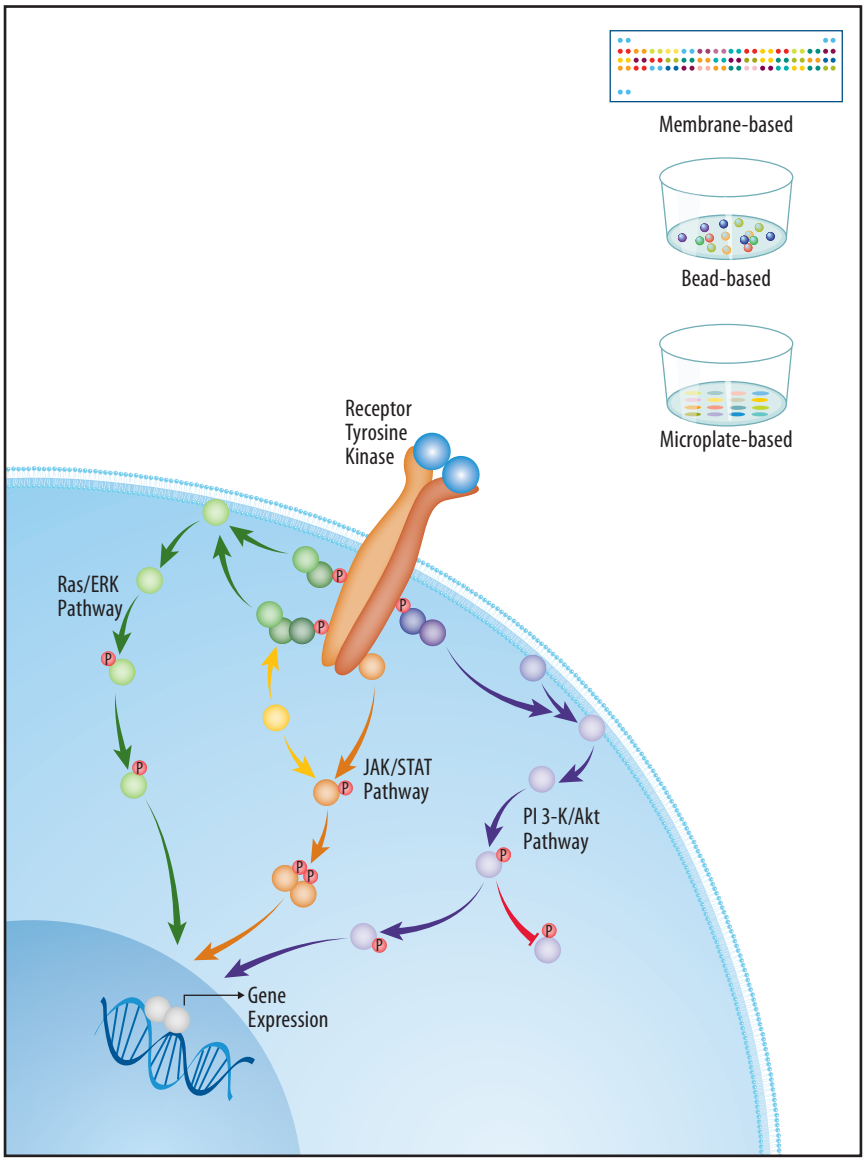
Multiplex Assays for Intracellular Factors

Multiplex Assays for Signal Transduction

Choose the Format that Meets Your Research Needs



R&D SYSTEMS®

WHAT FORMAT ARE YOU LOOKING FOR?

Multiplex Assays for Intracellular Factors: Choose the Format to Match Your Research Needs

Depending on your research goals, multianalyte profiling can provide a useful alternative to traditional Western blot or single analyte ELISA. Multianalyte assays can reveal changes in protein levels or phosphorylation in a larger context, providing a much better picture of the overall intracellular response to treatment. In addition, single analyte assays are impractical for rapidly screening large numbers of samples for drug discovery purposes. R&D Systems offers the most referenced multianalyte profiling tools for signal transduction. They are available in multiple formats to match your research needs.

Membrane-based Antibody Arrays

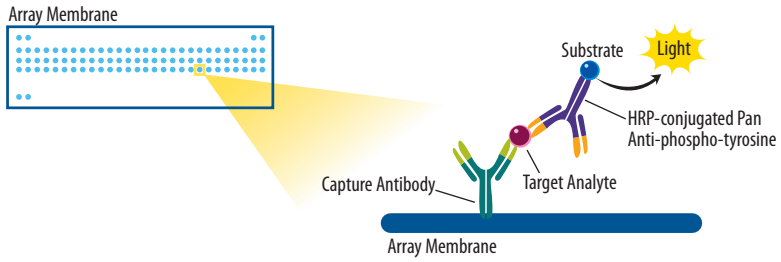
Proteome Profiler™ Antibody Arrays *Fast, Easy, and Without Specialized Equipment*

R&D Systems Proteome Profiler Antibody Arrays offer the widest panel of target molecules per array for sample screening. These macroarrays, which consist of up to 59 specific capture antibodies spotted in duplicate onto nitrocellulose membranes, are ideal for profiling multiple intracellular proteins in cell lysates. Arrays utilize standard chemiluminescent detection reagents and equipment, and chemiluminescence is detected in the same manner as a Western blot.

FEATURES

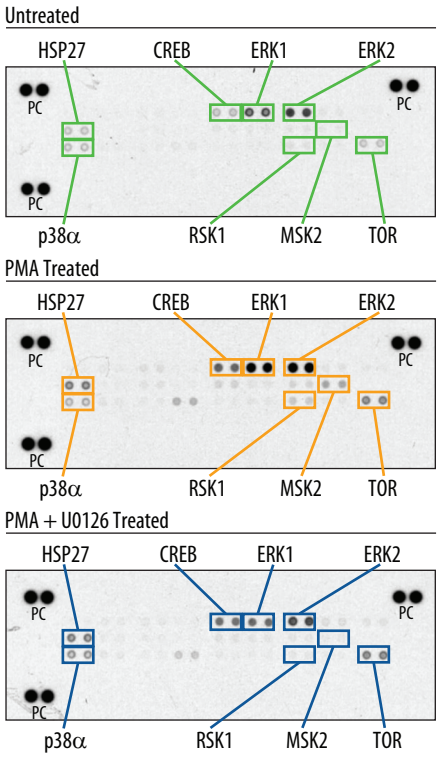
- ✓ Rapid screening of a large number of analytes
- ✓ Easy to use
- ✓ Requires no specialized equipment
- ✓ The most referenced phospho-antibody arrays

ASSAY PRINCIPLE

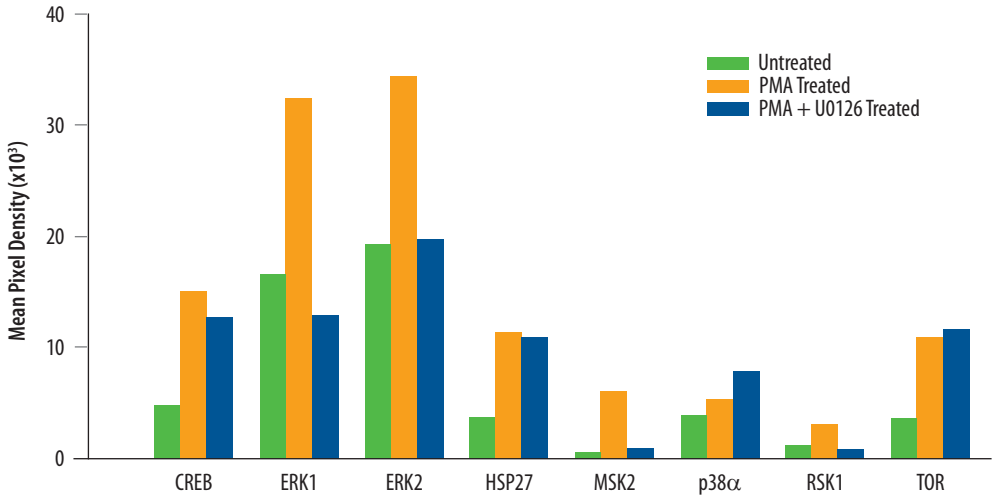


Proteome Profiler Array Assay Principle. Cell lysates are incubated with antibody-spotted nitrocellulose membranes. The immobilized antibodies on the membranes capture specific proteins present in the sample. Target proteins are detected using a cocktail of biotinylated detection antibodies and Streptavidin-HRP, or an HRP-conjugated pan anti-phospho-tyrosine antibody. Bound analytes are visualized with chemiluminescence in the same manner as a Western blot.

A.



B.

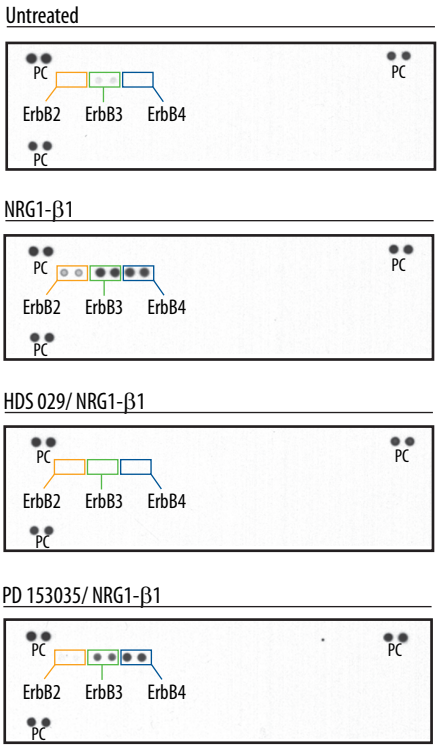


Assessment of Kinase Phosphorylation in HeLa Cells Following Treatment with PMA and U0126. A. The HeLa human cervical epithelial carcinoma cell line was untreated or treated with phorbol 12-myristate 13-acetate (PMA) either with or without the MEK inhibitor U0126 (Catalog # 1144). Cell lysates from untreated and treated cells were assessed for the phosphorylation of 26 intracellular kinases, including nine mitogen-activated protein kinases (MAPKs), using the Proteome Profiler Human Phospho-MAPK Antibody Array Kit (Catalog # ARY002B). **B.** Histogram profiles for select analytes were generated by analysis of the mean pixel density of individual antibody spots using image software analysis. PC=Positive Control.

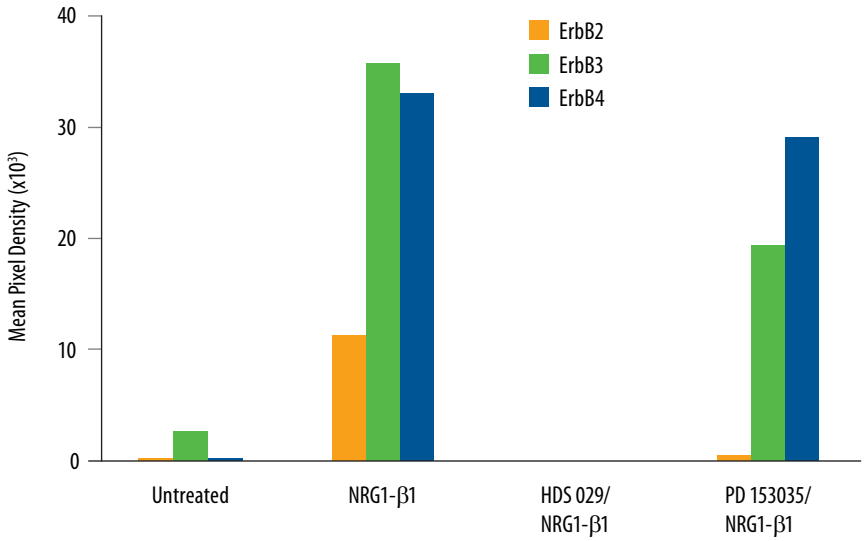
Proteome Profiler Antibody Arrays

Human Apoptosis Antibody Kit Catalog # ARY009
Bad, Bax, Bcl-2, Bcl-x, Cleaved Caspase-3, Pro-Caspase-3, Catalase, Claspin, Clusterin, Cytochrome c, FADD, Fas/TNFRSF6, HIF-1α, HO-1/HMOX1, HO-2/HMOX2, HSP27, HSP60, HSP70/HSPA1A, HTRA2/Omi, cIAP-1 (HIAP-2), cIAP-2 (HIAP-1), Livin, p21/CIP1/CDNK1A, p27/Kip1, Phospho-p53 (S15), Phospho-p53 (S46), Phospho-p53 (S392), PON2, Phospho-Rad17 (S635), SMAC/Diablo, Survivin, TNF RI, TRAIL R1, TRAIL R2, XIAP
Human Cell Stress Antibody Kit Catalog # ARY018
ADAMTS1, Bcl-2, Carbonic Anhydrase IX, Cited-2, COX-2, Cytochrome c, Dkk-4, FABP1/L-FABP, HIF-1α, HIF-2α, Phospho-HSP27 (S78/S82), HSP60, HSP70/HSPA1A, IDO, Phospho-JNK Pan (T183/Y185), NFκB1, p21/CIP1/CDNK1A, p27/Kip1, Phospho-p38α (T180/Y182), Phospho-p53 (S46), PON1, PON2, PON3, SIRT2, SOD2/Mn-SOD, Thioredoxin-1
Human Phospho-Immunoreceptor Antibody Kit Catalog # ARY004
2B4/CD244/SLAMF4, BLAME/SLAMF8, BTLA, CD3ε, CD5, CD6, CD23/Fcε RII, CD28, CD31/PECAM-1, CD84/SLAMF5, CD229/SLAMF3, CEACAM-1, CLEC-1, CLEC-2, CRACC/SLAMF7, CTLA-4, DCIR/CLEC4A, Dectin-1/CLEC7A, DNAM-1, Fcγ RIIA/CD32a, Fcγ RIIIA/B, FCRL1/FcRH1, FCRL2/FcRH2, FCRL4/FcRH4, FCRL5/FcRH5, ILT2/CD85j, ILT3/CD85k, ILT4/CD85d, ILT5/CD85a, ILT6/CD85e, Integrin β3/CD61, KIR2DL4/CD158d, LAIR1, LAIR2, LMIR1/CD300A/CMRF-35H, LMIR2/CD300c, LMIR3/IREM-1, LMIR6/IREM-2, MDL-1/CLEC5A, NKp30, NKp44, NKp46/NCR1, NKp80/KLRF1, NTB-A/SLAMF6, PD-1, SHIP, SHP-1, SHP-2, Siglec-2/CD22, Siglec-3/CD33, Siglec-5, Siglec-7/CD328, Siglec-9, Siglec-10, SIRPβ1/CD172b, SLAM/CD150, TREM-1, TREM-2, TREML1/TLT-1
Human Phospho-Kinase Antibody Kit Catalog # ARY003B
Akt (S473) Pan Specific, Akt (T308) Pan Specific, AMPKα1 (T174), AMPKα2 (T172), β-Catenin, Chk2 (T68), c-Jun (S63), CREB (S133), EGF R (Y1068), eNOS (S1177), ERK1 (T202/Y204)/ERK2 (T185/Y187), FAK (Y397), Fgr (Y412), Fyn (Y420), GSK-3α/β (S21/S9), Hck (Y411), HSP27 (S78/S82), HSP60, JNK (T183/Y185, T221/Y223) Pan Specific, Lck (Y394), Lyn (Y397), MSK1 (S376)/MSK2 (S360), p27/Kip1 (T198), p38α (T180/Y182), p53 (S15), p53 (S46), p53 (S392), p70 S6 Kinase (T229), p70 S6 Kinase (T389), PDGF Rβ (Y751), PLC-γ1 (Y783), PRA540 (T246), PYK2/FAK2 (Y402), RSK1 (S380)/RSK2 (S386)/RSK3 (S377), Src (Y419), STAT2 (Y689), STAT3 (Y705), STAT3 (S727), STAT5a (Y694), STAT5a/b (Y694/Y699), STAT5b (Y699), STAT6 (Y641), TOR (S2448), WNK-1 (T60), Yes (Y426)
Human Phospho-MAPK Antibody Kit Catalog # ARY002B
Akt1 (S473), Akt2 (S474), Akt3 (S472), Akt (S473, S474, S472) Pan Specific, CREB (S133), ERK1 (T202/Y204), ERK2 (T185/Y187), GSK-3α/β (S21/S9), GSK-3β (S9), HSP27 (S78/S82), JNK1 (T183/Y185), JNK2 (T183/Y185), JNK3 (T221/Y223), JNK (T183/Y185)/(T221/Y223) Pan Specific, MKK3 (S218/T222), MKK6 (S207/T211), MSK2/RSKB (S360), p38α (T180/Y182), p38β (T180/Y182), p38δ (T180/Y182), p38γ (T183/Y185), p53 (S46), p70 S6 Kinase (T421/S424), RSK1 (S380), RSK2 (S386), TOR (S2448)
Human Phospho-RTK Antibody Kit Catalog # ARY001B
ALK/CD246, Axl, DDR1, DDR2, Dtk, EGF R, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, ErbB2, ErbB3, ErbB4, FGF R1, FGF R2α, FGF R3, FGF R4, Flt-3/Flk-2, HGF R/c-MET, IGF-I R, Insulin R/CD220, M-CSF R, Mer, MSP R/Ron, MuSK, PDGF Rα, PDGF Rβ, Ret, ROR1, ROR2, Ryk, SCF R/c-kit, Tie-1, Tie-2, TrkA, TrkB, TrkC, VEGF R1/Flt-1, VEGF R2/KDR, VEGF R3/Flt-4
Human Pluripotent Stem Cell Antibody Kit Catalog # ARY010
E-Cadherin, α-Fetoprotein, GATA-4, Goosecoid, HCG, HNF-3β/FoxA2, Nanog, Oct-3/4, Otx2, PDX-1/IPF1, Snail, SOX2, SOX17, p63/TP73L, VEGF R2/KDR
Mouse Phospho-RTK Antibody Kit Catalog # ARY014
Axl, Dtk, EGF R, EphA1, EphA2, EphA3, EphA6, EphA7, EphA8, EphB1, EphB2, EphB4, EphB6, ErbB2, ErbB3, ErbB4, FGF R2 (IIIc), FGF R3, FGF R4, Flt-3/Flk-2, HGF R/c-MET, IGF-I R, Insulin R/CD220, M-CSF R, Mer, MSP R, MuSK, PDGF Rα, PDGF Rβ, c-Ret, SCF R/c-kit, Tie-1, Tie-2, TrkA, TrkB, TrkC, VEGF R1/Flt-1, VEGF R2/Flk-1, VEGF R3/Flt-4

A.



B.



Assessment of RTK Phosphorylation in Breast Cancer Cells. A. The MDA-MB-453 human breast cancer cell line was untreated or treated with the ErbB receptor family inhibitors HDS 029 (Catalog # 2646) or the EGF R inhibitor PD 153035 (Catalog # 1037). Cells were subsequently treated with Recombinant Human NRG1-β1/HRG1-β1 (Catalog # 396-HB) or remained untreated. Phosphorylation of 49 different RTKs in cell lysates was assessed using the Proteome Profiler Human Phospho-RTK Antibody Array Kit (Catalog # ARY001B). **B.** Histogram profiles for select analytes were generated by analysis of the mean pixel density of individual antibody spots using image software analysis. PC = Positive Control.

For more information, please visit our website at www.RnDSystems.com/ProteomeProfiler

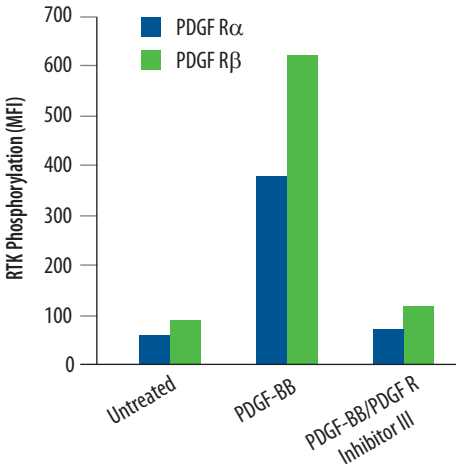
Bead-based Multianalyte Profiling

VersaMAP™ Magnetic Custom Multiplex System Kits *For the Luminex Platform*

R&D Systems VersaMAP Magnetic Custom Multiplex System Kits are homogeneous, no wash assays designed for the Luminex® platform. These assays utilize analyte-specific antibodies conjugated to superparamagnetic microparticles to simultaneously detect up to 25 user-defined phosphorylated receptor tyrosine kinases (RTKs) in a single sample of cell lysate. RTKs are chosen from a list of potential analytes using our specialized online ordering tool.

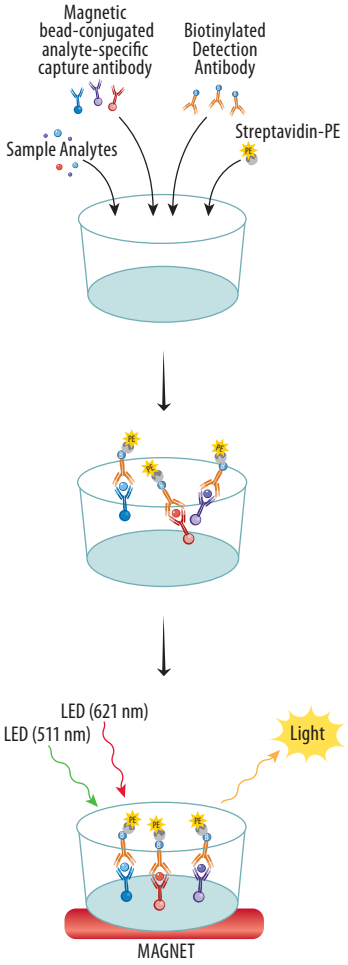
FEATURES

- ✓ User defines up to 25 analytes of interest
- ✓ Simultaneous detection of selected analytes
- ✓ Requires small sample size
- ✓ Homogeneous Assay: no washing required
- ✓ Compatible with multiple analyzing instruments*



Induction and Inhibition of PDGF Rα and PDGF Rβ Phosphorylation in NIH-3T3 Cells. The NIH-3T3 mouse embryonic fibroblastic cell line was untreated, treated with Recombinant Human PDGF-BB (Catalog # 220-BB), or pre-treated with PDGF-R Tyrosine Kinase Inhibitor III followed by Recombinant Human PDGF-BB. Phosphorylation of PDGF Rα and PDGF Rβ in cell lysates was assessed using the VersaMAP Magnetic Human RTK Multiplex System Kit B (Catalog # VMAPMAGB). Histogram profiles of PDGF Rα (blue bars) and PDGF Rβ (green bars) phosphorylation were generated by analysis of the median fluorescence index (MFI).

ASSAY PRINCIPLE



Step 1. The VersaMAP Magnetic Custom Multiplex System is a homogeneous, no wash assay. A reaction mixture containing capture antibodies immobilized on superparamagnetic microparticles, a biotinylated detection antibody, phycoerythrin (PE)-conjugated streptavidin, and sample is added to each well of a 96-well microplate.

Step 2. The immobilized analyte-specific capture antibodies bind both unphosphorylated and phosphorylated forms of the target RTKs present in the sample. The phosphorylated RTKs are detected by the biotinylated anti-phospho-tyrosine detection antibody and Streptavidin-PE.

Step 3. The beads are read using the Luminex MAGPIX Analyzer.* A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct LEDs illuminate the beads. One LED identifies the analyte that is being detected and the second LED determines the magnitude of the PE-derived signal. Each well is imaged with a CCD imager.

VersaMAP Magnetic Custom Multiplex System Kits

Select up to 25 analytes from one of the panels listed below.

Human RTK Multiplex System Kit A **Catalog # VMAPMAGA**
EGF R, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphB1, EphB2, EphB3, EphB4, EphB6, ErbB2, ErbB3, ErbB4, Flt-3/Flk-2, HGF R/c-MET, IGF-I R, M-CSF R, MSP R/Ron, Ret, SCF R/c-kit, TrkA
Human RTK Multiplex System Kit B **Catalog # VMAPMAGB**
ALK/CD246, Axl, CCK4, DDR1, DDR2, Dtk, FGF R1, FGF R2α, FGF R3, FGF R4, Insulin R/CD220, LTK, Mer, MuSK, PDGF Rα, PDGF Rβ, ROR1, ROR2, Tie-1, Tie-2, TrkB, TrkC, VEGF R1/Flt-1, VEGF R2/KDR, VEGF R3/Flt-4

For more information, please visit our website at www.RnDSystems.com/VersaMAPMagnetic

** VersaMAP Magnetic Custom Multiplex Kits are compatible with the Luminex® MAGPIX®, Luminex 100/200™, and Bio-Rad® Bio-Plex® analyzers.*

Microplate-based Antibody Arrays

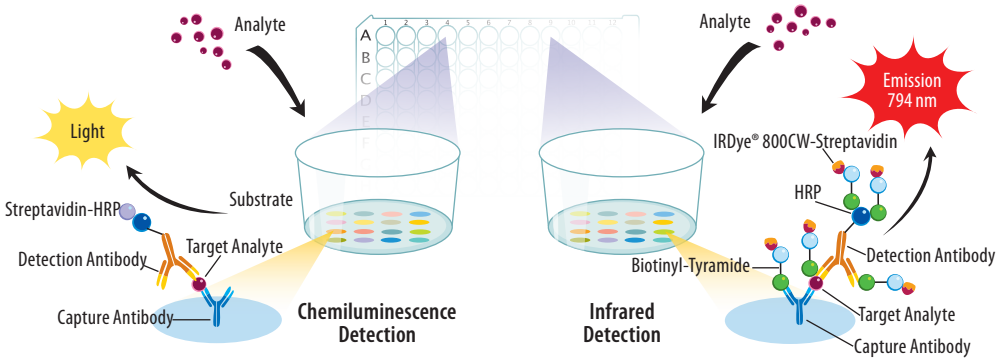
Proteome Profiler™ *96* Antibody Arrays *For High-throughput Analysis*

R&D Systems Proteome Profiler *96* Antibody Arrays are supplied as a 96-well microplate spotted with a focused selection of up to 16 capture antibodies, providing an antibody array in each well. These assays are used for measuring the relative phosphorylation of multiple intracellular signaling molecules in a single cell lysate sample. Kits are available for use with chemiluminescence detection and infrared-detection. They can be purchased as either a predefined kit or as a custom-designed kit that matches your specific research needs. Additionally, the potential for more than 1500 data points per microplate makes these assays ideal for high-throughput screening.

FEATURES

- ✓ An array in each well of a 96-well microplate
- ✓ Screen for multiple proteins in a single sample
- ✓ Up to 1536 data points per microplate
- ✓ Utilizes small sample size
- ✓ Fast and easy to use
- ✓ Amenable to high-throughput analysis
- ✓ Available in chemiluminescence and infrared detection formats
- ✓ Available as predefined or custom arrays

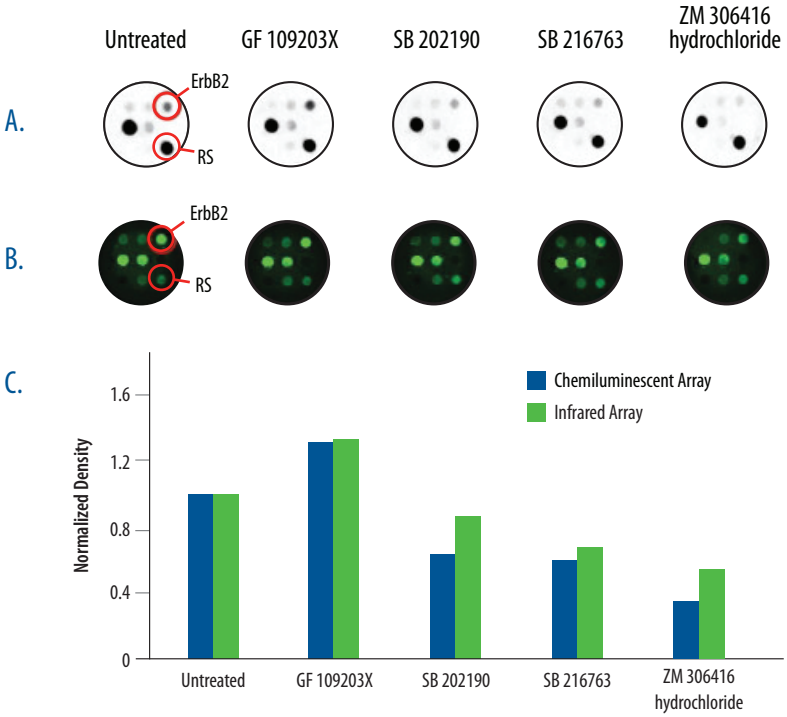
ASSAY PRINCIPLE



Proteome Profiler *96* Antibody Array Assay Principle. The Proteome Profiler *96* Antibody Arrays consist of a 96-well microplate spotted with a series of capture antibodies in each well. Target proteins present in cell lysates bind to the immobilized antibodies. Bound proteins are detected using a cocktail of biotinylated detection antibodies and Streptavidin-HRP, or an HRP-conjugated pan anti-phospho-tyrosine antibody. Bound analytes can be visualized with chemiluminescence and common digital imaging systems.** Bound analytes can also be visualized using IRDye® 800CW-conjugated Streptavidin[a] and a compatible near-infrared imager, such as the LI-COR Odyssey® Infrared Imaging System. For infrared detection of bound analytes, signals are amplified using biotinyl-tyramide[b] and hydrogen peroxide prior to the addition of the infrared dye.

Proteome Profiler *96* Microplate-based Antibody Arrays

Human Phospho-RTK Array 1 **Chemiluminescent Detection:** Catalog # ARZ001 **Infrared Detection:** Catalog # ARZ001NIR
EGF R, ErbB2, ErbB3, ErbB4, HGF R/c-MET, IGF-I R, Insulin R/CD220, M-CSF R, MSP R/Ron, PDGF Rα, PDGF Rβ, SCF R/c-kit, Tie-2, VEGF R1/Flt-1, VEGF R2/KDR, VEGF R3/Flt-4
Human Phospho-RTK Array 2 **Chemiluminescent Detection:** Catalog # ARZ002 **Infrared Detection:** Catalog # ARZ002NIR
EGF R, EphB4, ErbB2, ErbB3, ErbB4, HGF R/c-MET, IGF-I R, MSP R/Ron
Human Phospho-RTK Array 3 **Chemiluminescent Detection:** Catalog # ARZ003 **Infrared Detection:** Catalog # ARZ003NIR
EphB4, PDGF Rα, PDGF, Rβ, Tie-1, Tie-2, VEGF R1/Flt-1, VEGF R2/KDR, VEGF R3/Flt-4
Human Phospho-Kinase Array 1 **Chemiluminescent Detection:** Catalog # ARZ004
Akt (S473) Pan Specific, ERK1/ERK2 (T202/Y204), GSK-3β (S9), JNK (T183/Y185) Pan Specific, p38α (T180/Y182), p70 S6 Kinase (T421/S424), Src (Y416), HSP60



Screening Changes in RTK Phosphorylation using Pharmacological Inhibitors. The MDA-MB-453 human breast cancer cell line was treated with a library of protein kinase inhibitors (Catalog # 3514). Cell lysates were analyzed for the phosphorylation of 8 different receptor tyrosine kinases (RTKs) using the Proteome Profiler *96* Human Phospho-RTK Array 2 (**A**; Catalog # ARZ002) and the Proteome Profiler *96* Human Phospho-RTK Array 2, Infrared (**B**; Catalog # ARZ002NIR). The intensity of the phospho-ErbB2 signal in each well was assessed. **C**. Histogram profiles of normalized pixel and optical densities of phospho-ErbB2 signals obtained by chemiluminescent (blue bars) and infrared (green bars) detection, respectively, for the indicated protein kinase inhibitor treatments. Results obtained using the two Proteome Profiler *96* Antibody Array detection methods are comparable. RS = Reference Spot.

For more information, please visit our website at www.RnDSystems.com/ProteomeProfiler96

***Compatible imaging systems include Quansys Biosciences Q-View™ Imager; Alpha Innotech Fluorchem® HD2 and FC2; Bio-Rad VersaDoc™ 4000 or ChemiDoc™ XRS Fujifilm LAS-3000 or LAS-3000 Mini; Aushon Biosystems SearchLight®; Carestream™ Image Station 4000MM Pro.*

[a]This product is covered by one or more of the following US Patent numbers: US 6,995,274, US 7,504,089, PCT - WO0224815A1, and US Application 61/184,750 pending.

[b]This product is distributed and sold to the End-User for life science research and commercial applications, but not for diagnostic use. End-User does not have a right to resell or transfer the TSA™ reagent component(s) of this product either alone or as components of another product. Any use of this product other than for life science research and commercial applications is strictly prohibited.

Tell Us What You Need!

Let Us Customize Your Multiplex Assay

For more information, please visit our website at www.RnDSystems.com/CustomProteomeProfiler*96*

Proteome Profiler *96* Custom Antibody Arrays

R&D Systems Proteome Profiler *96* Phospho Kinase Custom Arrays allow researchers to design a microplate-based assay for assessing the phosphorylation of 3 to 8 user-defined Kinases in each well of a 96-well plate. Kinases are chosen from a list of potential analytes using our specialized online ordering tool. This tool allows you to:

1. Select the species of interest.

2. Select the kit of choice.

3. Select the analytes to be included in your custom multiplex kit.

STEP 1 Select a Species: Human

STEP 2 Select a Panel: Proteome Profiler 96 Human Phospho-Kinase Custom Array

STEP 3 Select from the available analytes listed below

SELECT UP TO 8 ANALYTES

- ☐ Akt (S473)
- ☐ AMPKα1 (T174)
- ☐ β-Catenin
- ☐ CREB (S133)
- ☑ eNOS (S1177)
- ☐ ERK1/ERK2 (T202/Y204, T185/Y187)
- ☐ PYK2/FAK2 (Y402)
- ☑ Src (Y419)
- ☐ STAT3 (Y705)
- ☑ STAT5b (Y699)
- ☐ p27/Kip1 (T198)
- ☐ p38α (T180/Y182)
- ☐ p53 (S15)
- ☐ p53 (S392)
- ☐ p53 (S46)
- ☑ p70 S6 Kinase (T421/S424)
- ☑ FAK (Y397)
- ☑ GSK-3 α/β (S21/S9)
- ☐ HSP27 (S78/S82)
- ☐ JNK (T183/Y185, T221/Y223)
- ☑ MKK6 (S207/T211)

ORDER SUMMARY

Catalog # ARZC02-07

Analytes Selected:

1. eNOS (S1177)
2. Src (Y419)
3. STAT5b (Y699)
4. p70 S6 Kinase (T421/S424)
5. FAK (Y397)
6. GSK-3 α/β (S21/S9)
7. MKK6 (S207/T211)

Online Ordering — ADD TO CART

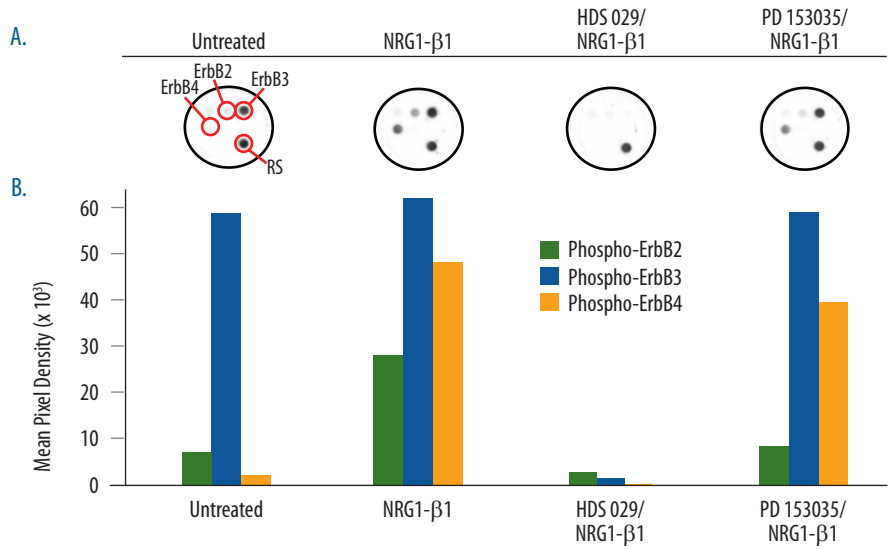
clear selections & start over — RESET

save selections & return later — SAVE

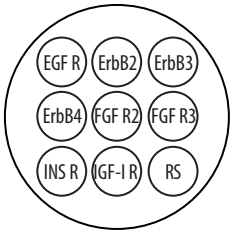
Proteome Profiler *96* Custom Antibody Arrays:

Select between 3 and 8 analytes from one of the panels listed below.

Human Phospho-RTK Array Kit Catalog # ARZC01	
ALK/CD246, Axl, DDR1, DDR2, Dtk, EGF R, EphA1, EphA2, EphA5, EphA10, EphB2, EphB3, EphB4, ErbB2, ErbB3, ErbB4, FGF R1, FGF R2α, FGF R3, FGF R4, Flt-3/Flk-2, HGF R/c-MET, IGF-I R, Insulin R/CD220, LMTK2, M-CSF R, Mer, MSP R/Ron, PDGF Rα, PDGF Rβ, Ret, Ryk, SCF R/c-kit, Tie-1, Tie-2, TrkA, TrkB, TrkC, VEGF R1/Flt-1, VEGF R2/KDR, VEGF R3/Flt-4	
Human Phospho-Kinase Array Kit Catalog # ARZC02	
Akt (S473) Pan Specific, AMPKα1 (T174), β-Catenin, CREB (S133), eNOS (S1177), ERK1 (T202/Y204)/ERK2 (T185/Y187), FAK (Y397), GSK-3 α/β (S21/S9), HSP27 (S78/S82), JNK (T183/Y185, T221/Y223) Pan Specific, MKK6 (S207/T211), p27/Kip1 (T198), p38α (T180/Y182), p53 (S392), p53 (S46), p53 (S15), p70 S6 Kinase (T421/S424), PYK2/FAK2 (Y402), Src (Y419), STAT3 (Y705), STAT5b (Y699)	
Mouse Phospho-RTK Array Kit Catalog # ARZC03	
Axl, Dtk, EGF R, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA8, EphB2, EphB3, EphB4, ErbB2, ErbB3, ErbB4, Flt-3/Flk-2, HGF R/c-MET, IGF-I R, Insulin R/CD220, M-CSF R, PDGF Rα, PDGF Rβ, Tie-1, Tie-2	



Well Map



Induction and Inhibition of RTK Phosphorylation in Breast Cancer Cells. A. The MDA-MB-453 human breast cancer cell line was untreated, treated with Recombinant Human NRG1-β1/HRG1-β1 (Catalog# 396-HB), or pretreated with ErbB inhibitor HDS 029 (Catalog # 2646) or the EGF R inhibitor PD 153035 (Catalog # 1037) followed by treatment with Recombinant Human NRG1-β1/HRG1-β1. Phosphorylation of 8 user-selected RTKs in cell lysates was assessed using the Proteome Profiler *96* Phospho-RTK Custom Array (Catalog # ARZC01). **B.** Histogram profiles for select RTKs were generated by analysis of the mean spot pixel densities. RS = Reference Spot.