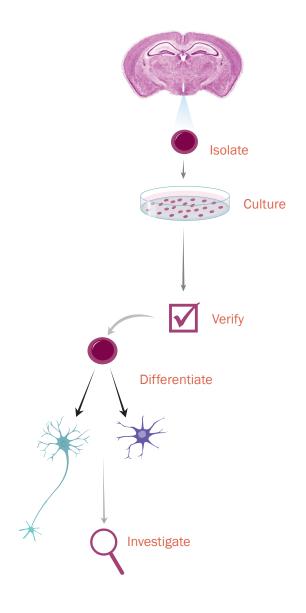
Neural Stem Cells



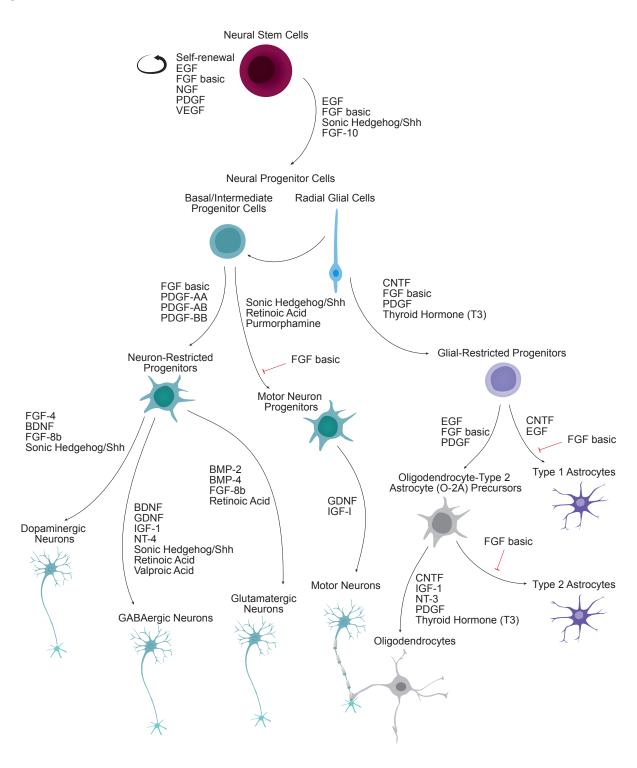






Neural Stem Cells

Neural stem cells (NSCs) are undifferentiated precursor cells defined by their capacity for self-renewal and mulitpotency. During central nervous system development, NSCs proliferate and divide to generate clonally related progeny that differentiate into neurons, astrocytes, oligodendrocytes, and ventricular ependymal cells. The symmetric division of NSCs underlies their ability to self-renew and serves to maintain the NSC population. In contrast, asymmetric mitosis produces one NSC and one neural progenitor cell (NPC), daughter cells with differentiation capacity restricted to neuronal or glial lineages. Asymmetric division generates two NPCs, but does not contribute to maintaining the NSC pool. NSC self-renewal and differentiation is regulated by a precise temporal sequence of growth factor presentation, intracellular signaling, and transcription factor expression. Bio-Techne presents tools to optimize NSC experimentation, from expansion and differentiation to verification and investigation. Using reagents that ensure a reliable workflow will reduce experimental variability, improve data consistency, and prevent wasted effort and reagents.



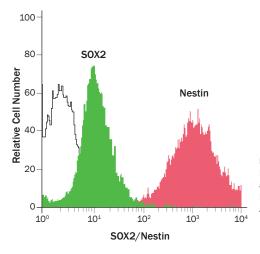
Isolate and Culture

NSCs require specialized media and growth factors to ensure efficient expansion. In addition to multipotent mouse and rat primary cortical stem cells, Bio-Techne offers a variety of serum-free neural media supplements, growth factors, and small molecules to maintain and expand NSCs.

Primary Cortical Stem Cells

- · Ready-to-Use-cells are highly pure and are contamination-free
- · Guaranteed Multipotency-confirmed differentiation into neurons, astrocytes, and oligodendrocytes
- Cost-Effective—use a homogeneous cell population to reduce experimental variation

Product	Description	Catalog #
Rat Primary Cortical Stem Cells	Isolated from E14.5 Sprague-Dawley rats; 3 x 10 ⁶ cells/vial	NSC001
Mouse Primary Cortical Stem Cells	Isolated from E14.5 CD-1 mice; 2 x 10 ⁶ cells/vial	NSC002



Mouse Cortical Stem Cells Express Nestin and SOX2. Mouse Cortical Stem Cells (Catalog # NSC002) express characteristic NSC markers, Nestin and SOX2. Cortical stem cells were cultured on Poly-L-ornithine and Fibronectin and were stained with a PE-Conjugated Mouse Anti-Mouse/Rat Nestin Monoclonal Antibody (red; Catalog # IC2736P), a PE-Conjugated Mouse Anti-Human/Mouse SOX2 Monoclonal Antibody (green; Catalog # IC2018P), or a PE-Conjugated Mouse IgG_{2A} Isotype Control Antibody (open; Catalog # IC003P).

Cell Culture Supplements and Substrates

- Improved Cell Health—high-quality culture reagents to ensure better growth and differentiation
- Efficient Growth—optimized to enhance neural cell growth in culture

Product	Catalog #
N-2 Plus Media Supplement	AR003
N-2 MAX Media Supplement	AR009
N21-MAX Media Supplement	AR008
Holo-Transferrin	2914-HT-001
StemXVivo™ Culture Matrix	CCM013
Human Fibronectin, CF	1918-FN-02M
Mouse Fibronectin, CF	1030-FN

Product	Catalog #
Recombinant Human Fibronectin Full, CF	4305-FN-200
Recombinant Human Fibronectin Fragment 2	3225-FN-100
Recombinant Human Fibronectin Fragment 3	3938-FN-050
Recombinant Human Fibronectin Fragment 4	3624-FN-050
Recombinant Human Fibronectin, ACFP	ACFP4305
Cultrex® Poly-L-Lysine	3438-100-01

Individual Growth Factors for NSC Expansion

- Clean Results—high purity and the lowest endotoxin levels on the market
- Consistent Performance—biological activity is equivalent across lots

Protein	Catalog #
Recombinant Human β-NGF	256-GF
Recombinant Human EGF	236-EG
Recombinant Human FGF basic	233-FB
Recombinant Human PDGF-AA	221-AA
Recombinant Human PDGF-BB	220-BB
Recombinant Human VEGF 165	293-VE

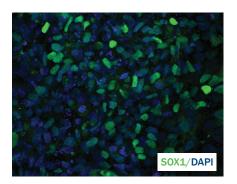
Protein	Catalog #
Recombinant Mouse β-NGF	1156-NG
Recombinant Mouse EGF	2028-EG
Recombinant Mouse FGF basic	3139-FB
Recombinant Mouse VEGF 164	493-MV

Protein	Catalog #
Recombinant Rat β-NGF	556-NG
Recombinant Rat EGF	3214-EG
Recombinant Rat FGF basic	3339-FB
Recombinant Rat PDGF-AA	1055-AA
Recombinant Rat PDGF-BB	520-BB
Recombinant Rat VEGF 164	564-RV

Tocris Small Molecules for NSC Expansion

Use small molecules alone or in combination with growth factors to enhance your control of NSC maintenance and expansion.

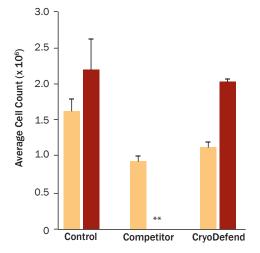
Product	Description	Catalog #
17-AAG	Selective Hsp90 inhibitor; protects neural progenitors from naturally occurring apoptosis	1515
Amiodarone hydrochloride	Ion channel blocker; selectively inhibits NSC proliferation in hESC-derived cell populations	4095
CHIR 99021	Selective GSK-3 inhibitor; enhances ESC self-renewal in combination with PD 0325901 (Catalog # 4192)	4423
Cyclopamine	Hedgehog signaling inhibitor; suppresses the proliferation of Ptch1+/- medulloblastoma precursor cells	1623
DMH-1	Selective ALK2 receptor inhibitor; promotes iPSC neurogenesis in combination with SB 431542 (Catalog # 1614)	4126
INDY	Dyrk1A/B inhibitor; impairs the self-renewal capacity of NSCs	4997
P7C3	Neuroprotective and proneurogenic compound; orally available	4076
SB 431542	Induces proliferation, differentiation and sheet formation of ESC-derived endothelial cells	1614
SU 5402	Potent FGFR and VEGFR inhibitor; attenuates integrin β4-mediated differentiation of neural stem cells	3300
Y-27632 dihydrochloride	Selective p160R0CK inhibitor; enhances survival of hES cells undergoing cryopreservation	1254



SB 431542 Promotes the Differentiation of Pluripotent Stem Cells into NSCs. BG01V human embryonic stem cells were grown to confluence then switched into differentiation media containing SB 431542 and Dorsomorphin. Differentiation into NSCs was confirmed at day 9 of differentiation by staining for SOX1 using the Goat Anti-Human SOX1 Affinity-Purified Polyclonal Antibody (Catalog #AF3369) and the NorthernLightsTM (NL)493-Conjugated Donkey Anti-Goat Secondary Antibody (Catalog #NL003).

CryoDefend™-Stem Cells Media

- Superior Recovery—high yields of viable NSCs compared to competitor media
- Reduced Variability—fully defined to ensure functionally consistent cells
- Better Control—protein-free formulation eliminates undefined factors commonly found in traditional cryopreservation media



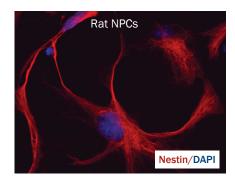
Superior Recovery of Rat NSCs from CryoDefend-Stem Cells Media Compared to Competitor Cryopreservation Media. Rat cortical stem cells (Catalog # NSC001) were frozen (2×10^6 cells/vial) in control (10% BSA/10% DMSO), competitor, or CryoDefend-Stem Cells media (Catalog # CCM018). Cell viability was assessed at the time of thaw (orange bars) and after 5 days of culture (red bars) in DMEM/F12 media supplemented with N-2 MAX (Catalog # AR009) and Recombinant Human FGF basic (20 ng/mL; Catalog # 41.14-TC). After 5 days in culture the number of viable cells recovered from cryopreservation in CryoDefend-Stem Cells was significantly increased compared to competitor (p < 0.001) media but not control (p = 0.637) media. Error bars indicate the standard deviation of duplicate samples. The asterisks (**) indicate low recovery yield.

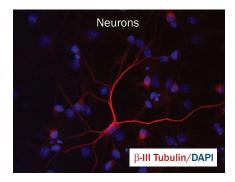
Verify

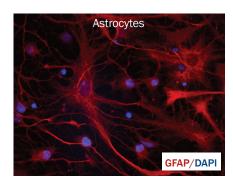
Validating the multipotency of a NPC population prior to their expansion, differentiation, and experimentation will ensure confidence when analyzing the data from downstream experiments. Using suboptimal, unverified NPC populations puts the investigator at risk for inconsistent results, thus wasting time and reagents. Bio-Techne offers a variety of solutions to quickly and confidently assess NPC multipotency.

Neural Lineage Functional Identification Kit

- Definitively Verifies Multipotency—uses optimized growth factors for unquestionable differentiation into neurons, astrocytes, and oligodendrocytes
- Time-Saving-differentiates NPCs in 7-10 days
- · Cost-Efficient—differentiation supplements and cell-specific marker antibodies packaged together





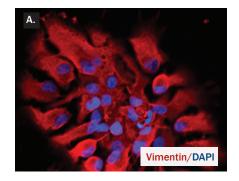




Functional Verification of NSC Multipotency. Rat cortical stem cells were maintained in culture and differentiated towards neural lineages using the Human/Mouse/Rat Neural Lineage Functional Identification Kit (Catalog # SC028). NSC differentiation into neurons, astrocytes, and oligodendrocytes was validated using the cell-specific marker antibodies supplied in the kit.

Neural Progenitor Cell Marker Antibody Panel

- Comprehensive Characterization—includes 8 antibodies against known NPC markers
- Trustworthy Results-combines multiple markers for accurate cell identification





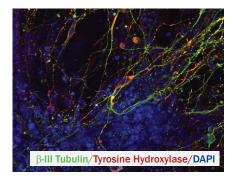
Verification of Neural Progenitor Marker Expression in Human NPCs. Human NPCs were assessed for expression of neural progenitor markers using the Human/Mouse/Rat Neural Progenitor Cell Marker Antibody Panel (Catalog # SC025). Human NSCs were stained with A) a Rat Anti-Vimentin Monoclonal Antibody followed by the NL557-Conjugated Goat Anti-Rat Secondary Antibody (red; Catalog # NL013) or B) a Goat Anti-SOX1 Antigen Affinity-Purified Polyclonal Antibody followed by the NL557-Conjugated Donkey Anti-Goat Secondary Antibody (red; Catalog # NL001). The nuclei were counterstained with DAPI (blue). Data courtesy of Dr. Sue Hu, University of Minnesota.

Differentiate

Efficient and consistent NSC differentiation is essential for maximizing research productivity, increasing data reliability, and reducing the cost and labor associated with lengthy differentiation protocols. Bio-Techne remedies these challenges by providing all-in-one kits specifically designed to differentiate ES/iPS cells into neuronal cell-types, including kits for the directed differentiation of dopaminergic neurons or oligodendrocytes. In addition, Bio-Techne offers a wide selection of small molecules to enhance NSC differentiation.

Human/Mouse Dopaminergic Neuron Differentiation Kit

- Reproducible Differentiation—includes optimized growth factors for the consistent differentiation of dopaminergic populations from ES/iPS cells
- Eliminates Variation—reliable differentiation for confidence during downstream data interpretation
- · Consistent Quality—Each kit validated to generate high quality dopaminergic neurons



Dopaminergic Neurons Generated from ES Cells Using the Dopaminergic Neuron Differentiation Kit. D3 mouse ES cells were differentiated into dopaminergic neurons using the Dopaminergic Neuron Differentiation Kit (Catalog # SC001B). A dopaminergic phenotype was confirmed using the Mouse Anti-Human Tyrosine Hydroxylase Monoclonal Antibody (red; Catalog # MAB7566) followed by the NL557-Conjugated Donkey Anti-Mouse Antigen Affinity-Purified Secondary Antibody (Catalog # NL007). Cells were counterstained with the NL637-Conjugated Mouse Anti-Neuron-specific β-III Tubulin (Clone Tuj-1) Monoclonal Antibody (green; Catalog #NL1195V). The nuclei were counterstained with DAPI (blue).

Mouse Oligodendrocyte Differentiation Kit

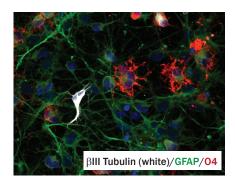
- Reproducible Differentiation—includes premium quality growth factors to efficiently differentiate ES/iPS cells into oligodendrocytes
- · Consistent Quality-each kit validated to generate high quality oligodendrocytes
- Cost-Saving—reliable differentiation to reduce experimental variation



Mouse Oligodendrocytes Generated Using the Mouse Oligodendrocyte Differentiation Kit. D3 mouse embryonic stem cells were expanded in KO-ES Media and differentiated into oligodendrocytes using the Mouse Oligodendrocyte Differentiation Kit (Catalog #SC004). An oligodendrocyte phenotype was detected using a Mouse Anti-Human/Mouse/Rat/Chicken Oligodendrocyte Marker O4 Monoclonal Antibody (red; Catalog # MAB1326) followed by staining with the NL557-Conjugated Affinity-Purified Goat Anti-Mouse Secondary Antibody (Catalog #NL019). The nuclei were counterstained with DAPI (blue).

Neural 3-Color Immunocytochemistry Kit

- Comprehensive Characterization—includes antibodies for neurons, astrocytes, and oligodendrocytes
- Time-Efficient—simultaneous 3-color staining with fluorochrome-conjugated primary antibodies



Differentiation of Rat NSCs Confirmed with the Neural 3-Color Immunocytochemistry Kit. Rat cortical stem cells were differentiated into neurons, astrocytes, and oligodendrocytes using the Human/Mouse/Rat Neural Lineage Functional Identification Kit (Catalog # SC028). Differentiated cells were stained with cell-specific antibodies from the Human/Mouse/ Rat Neural 3-Color Immunocytochemistry Kit (Catalog # SC024). The images show NSC cells differentiated into neurons (pseudocolored white; ßIII Tubulin), astrocytes (green; GFAP), and oligodendrocytes (red; O4). Nuclei were counterstained with DAPI (blue).

Individual Proteins for NSC Differentiation

Protein	Catalog #		
Protein	Human	Mouse	Rat
Recombinant BMP-2	355-BM	5020-BP	
Recombinant BMP-4	314-BP		
Recombinant CNTF	257-NT		557-NT
Recombinant FGF-10	345-FG	6224-FG	7804-FG
Recombinant GDNF	212-GD		512-GF
Recombinant IGF-I	291-G1	791-MG	4326-RG
Recombinant NT-3	267-N3		
Recombinant NT-4	268-N4	3236-N4	
Recombinant Sonic Hedgehog/Shh	1845-SH	464-SH	
Recombinant FGF-8b	423-F8	423-F8	
Recombinant BDNF	248-BD	248-BD	248-BD

Tocris Small Molecules for NSC Differentiation

Use small molecules to gain temporal control of differentiation pathways and to modulate cell fate by targeting specific signaling pathways.

Product	Description	Catalog #
DAPT	γ-secretase inhibitor; induces neuronal differentiation of neural cells	2634
Fluoxetine hydrochloride	5-HT re-uptake inhibitor; induces differentiation of neuronal precursors	0927
Forskolin	Adenylyl cyclase activator; induces neuronal differentiation in NSCs	1099
ISX 9	Induces neuronal differentiation of SVZ progenitors	4439
KHS 101 hydrochloride	Selective inducer of neuronal differentiation in hippocampal neural progenitors	4888
Metformin hydrochloride	Antidiabetic agent; promotes neurogenesis	2864
Neurodazine	Induces neurogenesis in mature skeletal muscle cells	3656
Neuropathiazol	Selective inducer of neuronal differentiation in hippocampal neural progenitors	5186
1-Oleoyl lysophosphatidic acid sodium salt	Endogenous agonist of LPA ₁ and LPA ₂ ; inhibits differentiation of NSCs into neurons	3854
O-Phospho-L-serine	Group III mGlu agonist; inhibits proliferation and enhances neuronal differentiation of progenitor cells	0238
Retinoic acid	Retinoic acid receptor agonist; promotes differentiation of ESCs into neurons, glia and adipocytes	0695
TCS 2210	Inducer of neuronal differentiation in MSCs	3877
TWS 119	GSK-3β inhibitor; induces neuronal differentiation in ESCs	3835
Valproic acid, sodium salt	Histone deacetylase inhibitor; promotes neuronal differentiation	2815

Investigate

After carefully validating NSC multipotency, expanding your NSC starting population, and driving cell differentiation, it is important to investigate the biology of newly formed cells. Bio-Techne offers a variety of tools to investigate neural function including Tocris small molecule agonists, antagonists, modulators, and blockers to functionally characterize the receptors and ion channels expressed by NSC-derived cells. In addition, explore synaptic development and function with our vast selection of neuroscience-related antibodies.

Tocris Small Molecules to Investigate Synapse Function

- Modulate neuronal function by targeting specific receptors and ion channels
- · Utilize our highly bioactive agonists, antagonists, modulators, and blockers

Product	roduct Description	
DL-AP5	Potent, selective NMDA antagonist	0105
(+)-Bicuculline	Potent GABA _A antagonist	0130
nor-Binaltorphimine dihydrochloride	Standard selective κ opioid receptor antagonist	0347
CGP 35348	Brain penetrant, selective GABA _B antagonist	1245
(+)-MK 801 maleate	Non-competitive NMDA antagonist, acts at ion channel site	0924
MPEP hydrochloride mGlu _s antagonist and positive allosteric modulator at mGlu ₄		1212
RS 127445 hydrochloride Selective, high affinity 5-HT _{2B} antagonist		2993
SCH 23390 hydrochloride Standard selective D ₁ -like antagonist; also 5-HT _{2c} agonist		0925
Tetrodotoxin	Na⁺ channel blocker	1078
(±)-U-50488 hydrochloride	Standard selective κ opioid receptor agonist	0495
Varenicline tartrate Orally active, subtype-selective α4β2 partial agonist		3754
Xanomeline oxalate	Functionally selective M ₁ agonist	3569

Learn more | www.tocris.com/Neuroscience

Antibodies to Investigate Synapse Development

Target Molecule Description		Applications	Species Reactivity	Catalog #	Brand
α-synuclein	Pre-synaptic protein	WB, FC, ICC, IHC	Human, Mouse	NBP1-26380	Novus
CaMKIIα	Concentrated at the postsynaptic density	WB, ELISA, ICC, IHC	Human, Mouse, Rat	NB100-1983	Novus
EAAT1/GLAST-1	Glutamate transporter	WB, IHC	Human	AF6048	R&D Systems
GFAP	Astrocyte marker	WB, IHC	Human	AF2594	R&D Systems
Homer1	Concentrated at the postsynaptic density	WB, ICC	Human, Mouse, Rat	NBP1-44999	Novus
MOG	Identifies myelinating oligodendrocytes	IHC	Human, Mouse	BAM2439	R&D Systems
Myelin basic protein Myelin sheath protein		WB	Human	MAB42281	R&D Systems
Neuroligin 1/NLGN1	leuroligin 1/NLGN1 Post-synaptic protein		Human, Rat	AF4340	R&D Systems
NMDAR2B NMDA receptor subunit		WB, FC, ICC, IHC	Human, Mouse, Rat	NB300-106	Novus
PSD95 Post-synaptic protein		WB, IHC	Human, Mouse, Rat	PPS059	R&D Systems
Shank3 Concentrated at the postsynaptic density		WB, IHC	Human, Mouse, Rat	NBP1-20197	Novus
Synapsin I Pre-synaptic protein		WB, IHC	Human, Mouse, Rat	PPS035	R&D Systems
Synaptophysin	ynaptophysin Neuronal secretory vescicle-associated protein		Human, Rat	AF5555	R&D Systems
Synaptotagmin	Synaptic vescicle protein		Human, Mouse, Rat	MAB4364	R&D Systems
Tyrosine Hydroxylase	Dopaminergic neuron marker	WB, ICC, IHC	Human, Mouse, Rat	NB300-109	Novus
vGLUT1 Glutamate transport into synaptic vesicles		WB, IHC	Human	NB110-57005	Novus

WB Western blot, FC Flow Cytometry, ICC Immunocytochemistry, IHC Immunohistochemistry

Learn more | novusbio.com/NeuralStemCells rndsystems.com/Antibodies

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