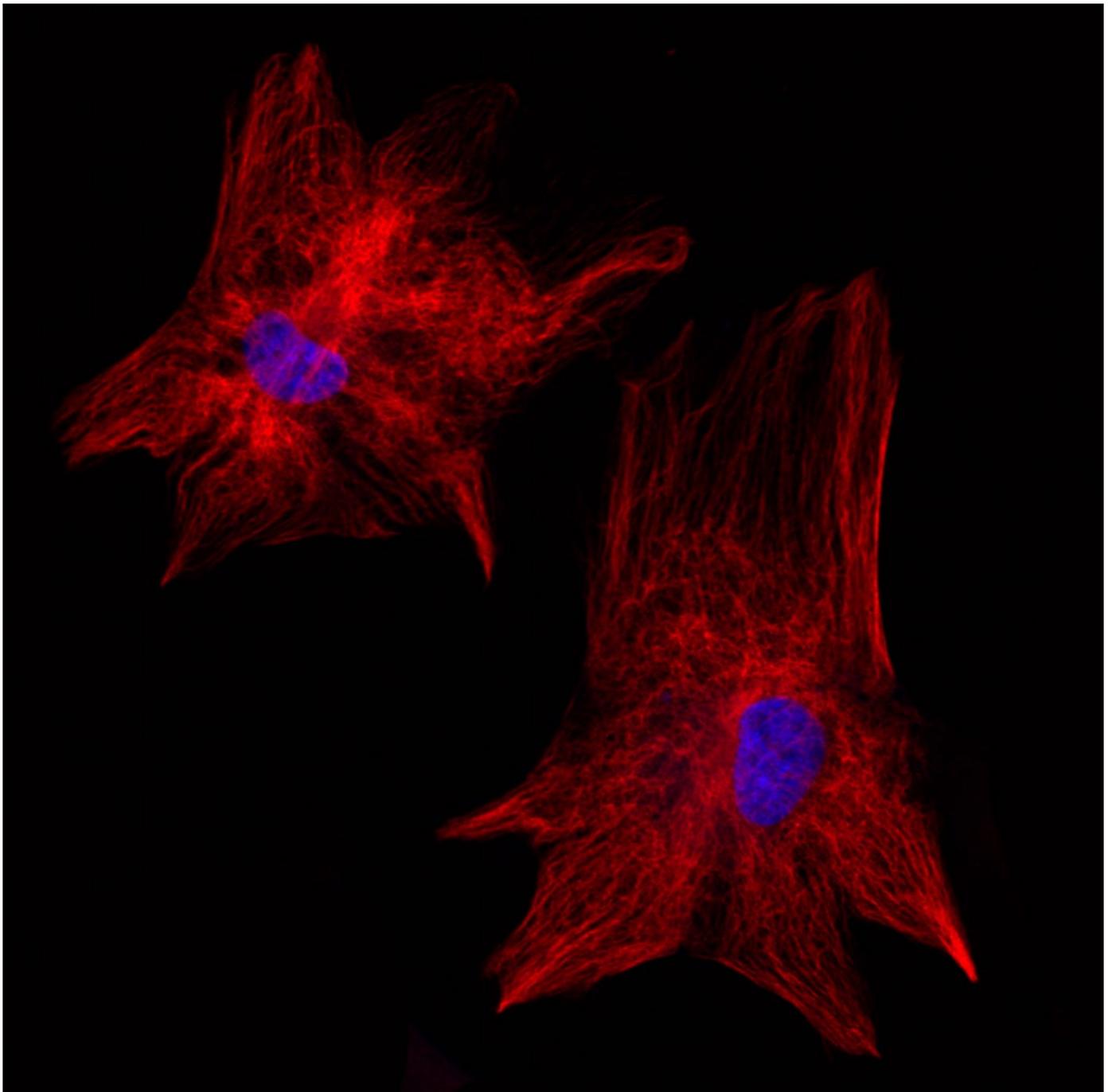


**biotechne®**

# Neural Stem Cell Workflow Solutions



# Isolate and Culture

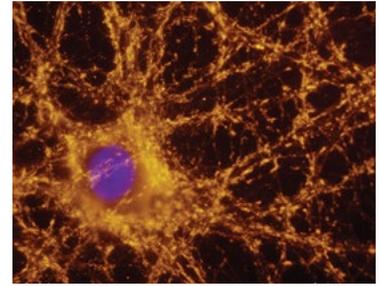
Neural Stem cells (NSCs) require specialized media and growth factors to ensure efficient expansion. In addition to multipotent mouse and rat primary cortical stem cells, Bio-Techne offers a variety of serum-free neural media supplements, growth factors, and small molecules to maintain and expand NSCs.

## Cell Culture

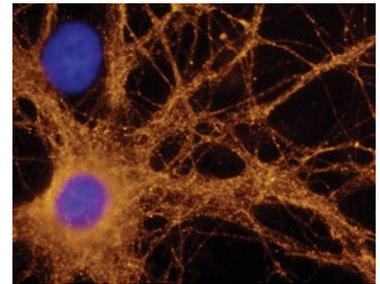
- **Improved Cell Health:** high-quality culture reagents to ensure better growth and differentiation
- **Efficient Growth:** optimized to enhance neural cell growth in culture

Product	Catalog #
GMP N-2 MAX Media Supplement <b>NEW!</b>	AR016
N-2 Plus Media Supplement	AR003
N-2 MAX Media Supplement	AR009
N21-MAX Media Supplement	AR008
N21-MAX Insulin Free Media Supplement	AR010
N21-MAX Vitamin A Free Media Supplement	AR012
Holo-Transferrin	2914-HT
Human Fibronectin, CF	1918-FN
Bovine Fibronectin, CF	1030-FN
Recombinant Human Fibronectin Full, CF	4305-FN
Recombinant Human Fibronectin Fragment 2	3225-FN
Recombinant Human Fibronectin Fragment 3	3938-FN
Recombinant Human Fibronectin Fragment 4	3624-FN
Recombinant Human Fibronectin, GMP	4305-GMP
Recombinant Human Fibronectin, ACFP	ACFP4305
Cultrex® Poly-L-Lysine	3438-100-01

N21-MAX



Competitor



**Increased Synaptic Puncta and Neurite Outgrowth of Primary Neurons Cultured in N21-MAX.** E18 rat hippocampal neurons were grown for 21 days *in vitro* in media supplemented with either N21-MAX Media Supplement (Catalog # AR008) or the neural media supplement from the most widely-used competitor. Staining for Synaptotagmin (yellow) showed more robust synaptic puncta and increased neurite outgrowth in neurons cultured in N21-MAX compared to those cultured in competitor media. Cells were stained with a Mouse Anti-Rat Synaptotagmin-1 Monoclonal Antibody (Catalog # MAB4364) followed by the NorthernLights™ (NL)557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL007). Nuclei were counterstained with DAPI (blue).

## Serum and Media for the Culture of Neural Stem Cells

Fetal bovine serum (FBS) is the ideal growth supplement for cell culture media and is generally preferred over other types of sera due to its high levels of nutrients and optimal combination of growth factors. Our FBS is produced utilizing strict manufacturing processes and quality assurance procedures, which guarantees unsurpassed quality and consistency from lot-to-lot. It is processed according to validated and approved cGMP and ISO 9001 guidelines and is globally sourced and collected in USDA-approved countries and documented to be exotic disease-free.

- Our Serum Testing Program enables the free screening of serum lots in your specific application
- Lot Matching Program assists in selecting serum based on the researcher's serum specifications and performance criteria

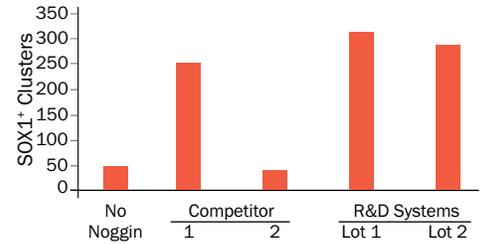


## Proteins for NSC Expansion and Differentiation

### Recombinant Proteins for Neural Stem Cell Culture and Differentiation

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

Recombinant Protein	Catalog #			ProDots Proteins, Catalog #
	Human	Mouse	Rat	Human
BDNF	248-BDB	248-BDB	248-BDB	
$\beta$ -NGF	256-GF	256-GF	556-NG	
BMP-2	355-BM	355-BM	355-BM	
BMP-4	314-BP	5020-BP		PRD314
CNTF	257-NT		557-NT	
EGF	236-EG	2028-EG	3214-EG	PRD236
FGF basic	233-FB	3139-FB	3339-FB	PRD233
FGF-4	235-F4	7486-F4		
FGF-8b	423-F8	423-F8		
GDNF	212-GD		512-GF	
IGF-1	291-G1	791-MG	4326-RG	PRD291
Noggin	6057-NG	1967-NG		PRD0657
NT-4	268-N4	3236-N4		
PDGF-BB	220-BB		520-BB	PRD220
Sonic Hedgehog (Shh)	8908-SH	464-SH		PRD-1845
VEGF 165	293-VE			PRD293



**Neuroectoderm Induction Using Recombinant Human Noggin.** BG01V human embryonic stem cells were driven into early cells of neuroectoderm using a 3 day incubation in recombinant human Noggin (25  $\mu$ g/mL) from either R&D Systems (Lot 1, Lot 2; Catalog # 6057-NG) or from two separate competitors (Competitor 1, Competitor 2). Control cells were not incubated in Noggin (No Noggin). The cells were stained for the neuroectoderm marker, SOX1 and SOX1<sup>+</sup> clusters were quantified under each of the indicated culture conditions. Cells treated with R&D Systems<sup>®</sup> Noggin showed an increase in SOX1<sup>+</sup> cells compared to both untreated and competitor-treated cells. R&D Systems<sup>®</sup> Noggin showed consistent differentiation across the lots tested.

## Tocris<sup>®</sup> 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	Primary Action	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 (Tocris, Catalog # 4192)	4423
Cyclopamine	Hedgehog signaling inhibitor; suppresses the proliferation of Ptch1 <sup>-/-</sup> medulloblastoma precursor cells	1623
DMH-1	Selective ALK2 receptor inhibitor; promotes iPSC neurogenesis in combination with SB 431542 (Tocris, 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
SB 203580	Selective inhibitor of p38 MAPK; stimulates NSC proliferation	1202
Y-27632 dihydrochloride	Selective p160ROCK inhibitor; enhances survival of hES cells undergoing cryopreservation	1254

## Primary Cortical Stem Cells and Cryopreservation Media

- **Ready-to-Use:** cells are highly pure and are contamination-free
- **Guaranteed Multipotency:** confirmed differentiation into neurons, astrocytes, and oligodendrocytes

Product	Primary Action	Catalog #
Rat Primary Cortical Stem Cells	Isolated from E14.5 Sprague-Dawley rats; $3 \times 10^6$ cells/vial	NSC001
Mouse Primary Cortical Stem Cells	Isolated from E14.5 CD-1 mice; $2 \times 10^6$ cells/vial	NSC002
CryoDefend <sup>®</sup> Stem Cells Media	For defined neural stem cell cryopreservation	CCM018

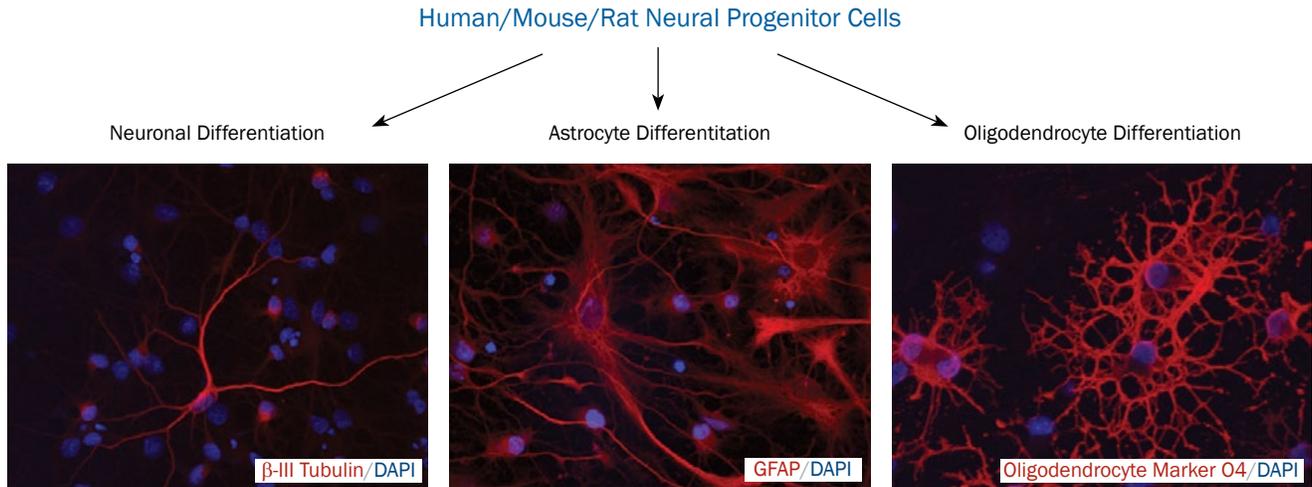
# Verify

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.

Product	Description	Catalog #
Neural Progenitor Cell Marker Antibody Panel	Human, Mouse, Rat	SC025
Neural Lineage Functional Identification Kit	Human, Mouse, Rat	SC028
Neural 3-Color Immunocytochemistry Kit	Human, Mouse, Rat	SC024

## Neural Lineage Functional Identification Kit

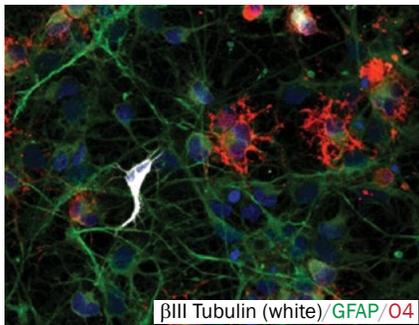
- **Definitively Verifies Multipotency:** uses optimized growth factors for unquestionable differentiation into neurons, astrocytes, and oligodendrocytes
- **Time-Saving:** differentiates NPCs in 7–10 days
- **Cost-Efficient:** differentiation supplements and cell-specific marker antibodies packaged together
- **Flexible:** for human, mouse, and rat NPCs



**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 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; βIII Tubulin), astrocytes (green; GFAP), and oligodendrocytes (red; O4). Nuclei were counterstained with DAPI (blue).

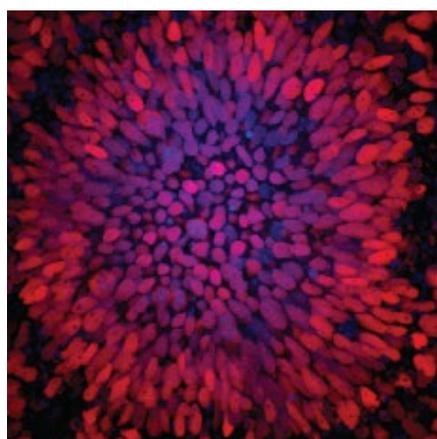
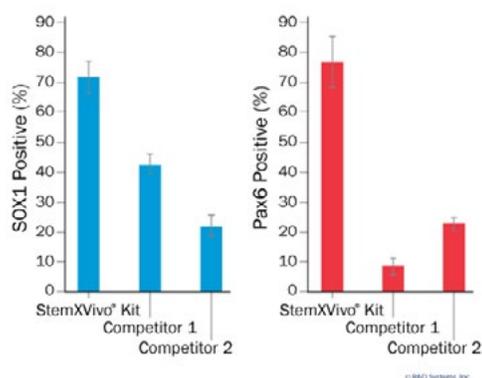
# Differentiate

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 embryonic stem (ES)/induced pluripotent stem (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.

## Differentiation Kits

- **Reproducible Differentiation:** includes optimized growth factors for the consistent differentiation of ES/iPS cells into dopaminergic neurons or oligodendrocytes
- **Eliminates Variation:** reliable differentiation for confidence during downstream data interpretation
- **Consistent Quality:** Each kit validated to generate high quality dopaminergic neurons

Product	Species	Catalog #
StemXVivo® Neural Progenitor Differentiation Kit	Human	SC035
Dopaminergic Differentiation Kit	Human, Mouse	SC001B
Oligodendrocytes Differentiation Kit	Mouse	SC004



## 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	$\gamma$ -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
LDN 193189	ALK2 and ALK3 inhibitor; induces differentiation of hPSCs into nociceptive sensory neurons	6053
Metformin hydrochloride	Antidiabetic agent; promotes neurogenesis	2864
Neuropathiazol	Selective inducer of neuronal differentiation in hippocampal neural progenitors	5186
1-Oleoyl lysophosphatidic acid sodium salt	Endogenous agonist of LPA <sub>1</sub> and LPA <sub>2</sub> ; inhibits differentiation of NSCs into neurons	3854
Retinoic acid	Retinoic acid receptor agonist; promotes differentiation of ESCs into neurons, glia and adipocytes	0695
SAG	Smo agonist; enhances differentiation of iPSCs into dopaminergic neurons	4366
SU 5402	FGFR and VEGFR inhibitor; attenuates integrin B4-induced differentiation of ESCs	3300
TWS 119	GSK-3 $\beta$ 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	Description	Catalog #
A 803467	Selective Na <sub>v</sub> 1.8 channel blocker	2976
α, β-Methyleneadenosine 5' triphosphate	P2 agonist	3209
DL-AP5	Potent, selective NMDA antagonist	0105
(+)-Bicuculline	Potent GABA <sub>A</sub> antagonist	0130
<i>nor</i> -Binaltorphimine dihydrochloride	Standard selective κ opioid receptor antagonist	0347
CGP 35348	Brain penetrant, selective GABA <sub>B</sub> antagonist	1245
(+)-MK 801 maleate	Non-competitive NMDA antagonist, acts at ion channel site	0924
MPEP hydrochloride	mGlu <sub>5</sub> antagonist and positive allosteric modulator at mGlu <sub>4</sub>	1212
RS 127445 hydrochloride	Selective, high affinity 5-HT <sub>2B</sub> antagonist	2993
SCH 23390 hydrochloride	Standard selective D <sub>1</sub> -like antagonist; also 5-HT <sub>2C</sub> agonist	0925
Tetrodotoxin	Na <sup>+</sup> 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 <sub>1</sub> agonist	3569

## Caged Compounds for Neural Cell Cultures

Product	Description	Catalog #
DPNI-caged-GABA	Nitroindoline-caged GABA	2991
MNI-caged-D-aspartate	Caged D-aspartate	2277
MNI-caged-L-glutamate	Stable photoreleaser of L-glutamate	1490
MNI-caged-NMDA	Caged NMDA	2224
NPEC-caged-(S)-AMPA	Caged (S)-AMPA	3840
NPEC-caged-D-AP5	Caged D-AP5 (Cat.No. 0106)	4230
NPEC-caged-dopamine	Caged dopamine	3992
RuBi GABA trimethylphosphine	Caged GABA; inhibits neural activity	4709
RuBi-Dopa	Caged dopamine; exhibits two-photon sensitivity	4932
RuBi-Glutamate	Caged glutamate; excited by visible wavelengths	3574
RuBi-Nicotine	Caged nicotine; rapidly excitable by visible light	3855

## Photoswitchable Ligands

Product	Description	Catalog #
AAQ	Photoswitchable K <sub>v</sub> channel blocker	5462
PA1	Photoswitchable ENa C blocker	5463
QAQ	Photoswitchable Na <sub>v</sub> , K <sub>v</sub> , and Ca <sub>v</sub> channel blocker	5470

## Fluorescent Probes for Neuroscience Research

Target Molecule	Description	Catalog #
Calcein AM	Cell permeable compound; hydrolyzed to become fluorescent in living cells	5119
Dansyl-NECA	Potent and selective fluorescent adenosine A1 agonist	5122
FFN 102 mesylate	Selective fluorescent substrate of DAT and VMAT2	5200
FFN 206 dihydrochloride	Fluorescent VMAT2 substrate	5043
FFN 511	Fluorescent substrate for VMAT2	3878
FURA-2AM	Fluorescent Ca <sup>2+</sup> indicator	2220
K 114	Amyloid fibril-specific fluorescent dye	3144
L 012 sodium salt	Chemiluminescent ROS and RNS indicator	5085
Methoxy-X04	Fluorescent amyloid $\beta$ detector; brain penetrant	4920
SynaptoRed™ C2	Fluorescent dye; stains synaptic vesicles	5118
Tocrifluor T1117	Novel fluorescent cannabinoid ligand; fluorescent form of AM 251 (Cat. No. 1117)	2540

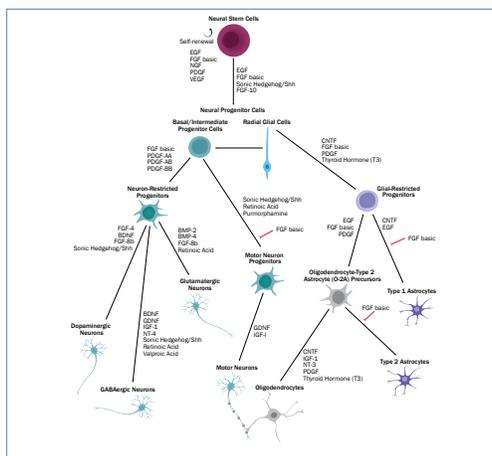
## Antibodies to Investigate Synapse Development

Target Molecule	Description	Applications	Species Reactivity	Catalog #	Brand
$\alpha$ -Synuclein	Pre-synaptic protein	WB, FC, ICC, IHC	Human, Mouse	NBP1-26380	Novus Biologicals
CaMKII $\alpha$	Concentrated at the postsynaptic density	WB, ELISA, ICC, IHC	Human, Mouse, Rat, +	NB100-1983	Novus Biologicals
EAAT1/GLAST-1	Glutamate transporter	WB, IHC	Human	AF6048	R&D Systems
GFAP	Astrocyte marker	WB, IHC, SW	Human	AF2594	R&D Systems
Homer1	Concentrated at the postsynaptic density	WB, ICC	Human, Mouse	NBP1-44999	Novus Biologicals
MOG	Identifies myelinating oligodendrocytes	IHC, WB	Mouse	AF2439	R&D Systems
Myelin basic protein	Myelin sheath protein	WB, ELISA, FC, ICC, IHC	Human	NBP2-22121	Novus Biologicals
Neurologin 1/NLGN1	Post-synaptic protein	WB, IHC	Human, Rat	AF4340	R&D Systems
NMDAR2B	NMDA receptor subunit	WB, FC, ICC, IHC, IP	Human, Mouse, Rat	NB300-106	Novus Biologicals
PSD95	Post-synaptic protein	WB	Human, Mouse, Rat	PPS059	R&D Systems
SHANK3	Concentrated at the postsynaptic density	WB, IHC	Human, Mouse, Rat	NBP1-47610	Novus Biologicals
Synapsin I	Pre-synaptic protein	WB, ICC, IHC, IP	Human, Mouse, Rat, +	PPS035	R&D Systems
Synaptophysin	Neuronal secretory vesicle-associated protein	WB, IHC	Human	AF5555	R&D Systems
Synaptotagmin	Synaptic vesicle protein	WB, ICC, IHC, IP	Rat	MAB4364	R&D Systems
Tyrosine Hydroxylase	Dopaminergic neuron marker	WB, ICC, IHC, SW	Human, Mouse, Rat, +	NB300-109	Novus Biologicals
Glut1	Glutamate transport into synaptic vesicles	WB, CHIP, FC, ICC, IHC	Human, Mouse	NB110-39113	Novus Biologicals

Species Key: + Additional Species

Applications Key: **WB** Western blot, **ChIP** Chromatin Immunoprecipitation, **FC** Flow Cytometry, **ICC** Immunocytochemistry, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **SW** Simple Western™

## View Pathway



[rndsystems.com/pathways\\_nsc](https://rndsystems.com/pathways_nsc)

## Neural Stem Cells

Neural stem cells (NSCs) are undifferentiated precursor cells defined by their capacity for self-renewal and multipotency. 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.

R&D SYSTEMS

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TOCRIS

protein simple

ACD

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