

Product Number(s): NP37100

NEUROMICS



RAT NEURAL STEM CELL KIT

Instruction Manual

This product is manufactured for Neuromics by Orion Biosolutions, Inc.

RELATED ANTIBODIES

Name	Catalog #	Type
A2B5	MO15016	Mouse IgM
GFAP	MO19001	Mouse IgG
GFAP	CH22102	Chicken IgY
MAP2	CH22103	Chicken IgY
Mash-1	MO15048	Mat IgG
Musashi-1	RA14128	Rabbit IgG
Nestin	GT15114	Goat IgG
NF-H	CH22104	Chicken IgY
NF-H, phosphorylated	MO22103	Mouse IgG
NF-L	CH22105	Chicken IgY
NF-L	MO22104	Mouse IgG
NF-M	CH22106	Chicken IgY
NF-M	MO22105	Mouse IgG
NF66	CH22101	Chicken IgY
Nucleostemin	GT15050	Goat IgG
Oct3/4	GT15052	Goat IgG
Oligodendrocyte Marker O1	MO15001	Mouse IgM
Oligodendrocyte Marker O4	MO15002	Mouse IgM
Pax 6	MO15017	Mouse IgG
Pax 7	MO15020	Mouse IgG
Sonic Hedgehog	GT15032	Goat IgG
Tyrosine Hydroxylase	MO20001	Mouse IgG

RELATED MEDIA

Name	Catalog #
Commitment Media	M37102
Minimal media for supplement addition	
Proliferation Media	M37101
Specially formulated for NSC expansion	

RELATED PROTEINS AND SUPPLEMENTS

Name	Catalog #
EGF, carrier-free	PR15005CF
FGF basic (146 aa)	PR15002
FGF basic (146 aa), carrier-free	PR15002CF
FGF basic (157 aa)	PR15003
FGF basic (157 aa), carrier-free	PR15003CF
FGF-8b	PR15004
FGF-8b, carrier-free	PR15004CF
Fibronectin, carrier-free	PR15001CF
GDNF (Human)	PR15007
GDNF (Human), Carrier Free	PR15007CF
GDNF (Rat)	PR15008
GDNF (Rat), Carrier Free	PR15008CF
N-2 Plus	S15101
Sonic Hedgehog	PR15000

RAT NEURAL STEM CELL KIT DESCRIPTION

Neuromics' *Rat Neural Stem Cell Kits* (Catalog # NP37100) are intended for the *in vitro* proliferation and commitment of rat neural progenitor cells to neuronal and/or glial lineages. The kit includes neural stem cells (NSCs), media to grow and proliferate the neural stem cells, and a minimal media to which growth factors can be added to induce differentiation. The proprietary *Proliferation Medium* comes complete with all the necessary supplements for culturing and expanding the rat NSCs, while the *Commitment Medium* provides an optimized minimal medium for addition of your own factors and supplements for differentiation.

KIT COMPONENTS

- Frozen vial (approximately 3×10^6 viable cells) of rat NSCs
 - ***Store in liquid nitrogen below -140°C***
- Proliferation Medium (Cat # M37101) with FGF-2 and proprietary ECM, 100 mls sterile-filtered
 - ***Store at -20°C***
- Commitment Medium (Cat # M37102) with 1uM retinoic acid/0.2% FBS, 100 mls sterile-filtered
 - ***Store at -20°C***

QUALITY CONTROL

A vial from each lot of *Rat Neural Stem Cell Kit* is thawed and cultured for 3-5 days following the protocol below. Tests are conducted for sterility (negative), cell proliferation (positive), and nestin expression (>80%) by intracellular FACS.

PROTOCOL OUTLINE

Pre-equilibrate Proliferation Media

- Incubate Proliferation Media in flask/dish for 1-3 hours in 37°C incubator prior to adding NSCs.

Prepare Neural Stem Cells

- Thaw cryovial in 37°C water bath by gently swirling for 2-3 minutes.
- Add cryovial contents to 10 mls of pre-warmed (37°C) Proliferation Medium and spin down.
- Discard supernatant and GENTLY resuspend NSCs in 2 mls Proliferation Medium and count viable cells.

Proliferate

- Add cells at desired density to flask/dish containing pre-equilibrated Proliferation Media.
- After 3-5 days feed cells with Proliferation Medium, pre-warmed to 37°C.
- Culture for an additional time as desired to obtain required cell density and phenotype.

Differentiate

- When ready to begin lineage commitment and differentiation, carefully aspirate Proliferation Medium and add Commitment Medium, pre-warmed to 37°C.
- Culture for an additional time in Commitment Media with selected supplements or factors to obtain required cell density and phenotype.

DETAILED PROTOCOL

Proper sterile Cell Culture Technique is required when working with the Neural Stem Cells

1. **Place cryovial in liquid nitrogen immediately upon receipt.** Be sure that the vial arrives with solid dry ice within the shipping container.
2. **Place Proliferation Medium and Commitment Medium in fridge at approximately 4°C.** If you are not going to start your experiment right away, store both media bottles in -20°C until the day before use, and then thaw overnight in fridge. Medium should be pre-warmed to approx. 37°C.
3. **Pre-equilibrate Proliferation Medium in 5-10% CO₂ 37°C incubator for 1-3 hours.** This step also allows for the proprietary ECM proteins in Proliferation Media to coat the flask or dish.
4. **Thaw cryovial in 37°C water bath by gentle swirling for 2-3 minutes.** Be sure to sterilize the outside of the vial with 70% alcohol before and after thawing.
5. **Immediately transfer vial contents into 15 ml sterile conical centrifuge tube with 10 mls of pre-warmed Proliferation Media.** Transfer GENTLY by filling pipet tip within cryovial, gently pipetting up and down to mix, then placing tip into medium within the conical tube and ejecting directly and slowly.
6. **Centrifuge at approximately 1,000 rpm/ 200g for 5 minutes, aspirate supernatant, and resuspend cell pellet in 2 mls Proliferation Medium.** Count in hemacytometer with 0.04% trypan blue dye. Each vial should yield approximately 3×10^6 viable cells.
7. **Add cells to flask/dish, using the table below as a guideline.** These volumes are approximate and each user should determine the optimal plating density for their specific needs. For example, if you want to analyze at near clonal density then you should reduce the volume or add to a dish with more surface area. Also, if you want to obtain higher cell numbers, you can extend the time period in Proliferation Medium in addition to plating at a lower density. **WARNING:** density can affect phenotype and state of differentiation. There are approximately 3×10^6 Cells viable cells/ cryovial which gives a final concentration of approx. 1.5×10^6 cells/ml.

Culture Dish	Volume to Plate	Yield / Vial	Total Volume
4-well chamber slide	0.025 mls to 0.05 mls/ well	40 to 80 wells	1.0 ml
6-well petri dish	0.125 to 0.25 mls/ well	10 to 20 wells	4 mls
10 cm Petri Dish/ T-75 cm ² flask	0.5 mls to 1.0 mls	2 to 4 wells or flasks	15 mls
T-150 cm ² flask	1.0 mls to 2.0 mls	1 to 2 flasks	20 mls

8. **Mix cells with media in flask/dish GENTLY by swirling by hand once or twice.** Be careful not to compromise sterility.
9. **After at least 3-5 days in culture in Proliferation Medium, GENTLY feed by addition of fresh Proliferation Medium pre-warmed to 37°C.** Aspiration is not recommended. Cells adhere loosely within the first 3-5 days, but cells that have not adhered after this time can be discarded if desired. Aspirate if desired VERY GENTLY. We recommend using a P200 tip over the end of the aspiration pipet. We use 20 mls of Proliferation Medium in a 150 cm² flask and feed 5 mls after 3-5 days.

DETAILED PROTOCOL (CONTINUED)

- 10. Culture cells for an additional time period in Proliferation Medium until the desired density has been reached, feeding with additional fresh Proliferation Medium every 3-5 days.** The additional growth can be extended for as long as your individual assay or phenotype requires. We generally allow another 3-10 days in Proliferation Medium. Remember that density can affect the degree of differentiation - in general higher densities will favor neuronal differentiation.

- 11. To begin the process of lineage commitment, aspirate Proliferation Medium GENTLY and add Commitment Medium, pre-warmed to 37°C.** The Commitment Medium contains 0.1% pre-qualified FBS and 1uM retinoic acid, which in addition to growth factor withdrawal, accelerates the process of lineage commitment and reduces the rate of proliferation/self-renewal. This minimal medium provides an effective “baseline” of lineage commitment and differentiation of neural stem and progenitor cells within the total population, however, each user should expect to add either known trophic factors (e.g. BDNF, NT’s, NGF, etc. for neurons), or your own experimental test compounds.