# NeuroStem™

## Fresh Rat Neuronal Progenitor Cells

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| Catalog # | Content | Amount |
|---|---|---|
| N300300 | Rat Hippocampal/Cortical Progenitor Cells | 3 million/2 ml |
| | NeuroPrep™ Media* | 12 ml |
| | Papain, Cell Culture Grade | 25 mg |
| | Neurobasal™/B27-Vitamin A/0.5 mM GlutaMAX™/Growth Factors Media | 12 ml |

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| **Shipping** | Shipped on blue ice. |
| **Storage** | **Store at 4°C for a few days; culture as soon as possible.** |

| RELATED PRODUCTS | Catalog # |
|---|---|
| NeuroPrep™ Media, 100 ml | NM100100 |
| Papain, Cell Culture Grade, 100 mg | NM100200 |
| NeuroPure™ Rat Hippocampal Cells, 1 million | N100200 |
| NeuroPure™ Rat Cortical Cells, 2 million | N200200 |
| NeuroPure™ P8 Rat Cerebellar Cells, 1 million | N300200 |
| NeuroPure™ E18 Primary Rat Hypothalamus Cells, 1 million | N400200 |
| NeuroPure™ E18 Primary Rat Striatum Cells, 1 million | N500200 |
| NeuroPure™ E18 Primary Rat Spinal Cord Cells, 1 million | N600200 |

**INTRODUCTION**: Neuroprogenitors or neural stem cells are brain cells that can multiply for many generations under appropriate culture conditions that include trophic growth factors and low adhesion. In culture with low adhesion substrates, they form neurospheres that can be dissociated for further expansion or differentiated. They are also pluripotent, meaning they are able differentiate into neurons, astroglia or oligodendrocytes. Potential uses include analysis of factors that influence either clonal yield or mass production. Other uses include preparation for grafting to repair damaged brain tissue and as carriers for introduction of specific genes into the brain.

## MATERIALS AND METHODS

### A-MEDIA PREPARATION

1. Prepare **NeuroPrep Papain Media (NPM)** by adding 5 mg papain to 2.5 ml of NeuroPrep™ Media. Mix for 15 minutes at 37°C, then filter sterilize using a 0.2 μm filter. Keep on ice in 15 ml tube until ready for use.

   **NOTE**: Prepare NPM fresh every time before use.

### B-CELL HANDLING AND GROWTH

2. Remove 1 ml of medium without disturbing brain tissue in tube and save in a 15 ml tube for use in Step 5 below.
3. Transfer brain tissue with a 2 ml pipette with as little medium as possible into the 15 ml tube containing NPM (Step 1 above).
4. Incubate tube in a 30°C water bath for 20 minutes; swirl every two minutes by hand.
5. With a sterile 9-inch Pasteur pipette (with a slightly fire-polished tip), transfer tissue into the 15 ml tube with saved media (Step 2). Triturate until most pieces of tissue are dispersed but for no more than 10 times and without creating any bubbles.
6. Let undispersed pieces settle by gravity for 1 minute.
7. Transfer supernatant to a new sterile 15 ml tube.
8. Spin at 1,100 rpm (200 x G) for 1 min. Discard supernatant.
9. Flick the tube to disperse the pellets of cells. Resuspend pellets in 2 ml of Neurobasal™/B27-Vitamin A/0.5 mM GlutaMAX™/Growth Factors media.
10. To determine density, aliquot 20 μl from Step 8 into 80 μl of a 0.4% trypan blue solution. Count cells using a hemacytometer.

    **NOTE**: The expected cell count is ~3 x $10^6$ cells (~$10^6$ from the hippocampal tissue and ~2 x $10^6$ cells from the cortical tissue).
11. Dilute cells to 150,000 cells/ml using Neurobasal™/B27-Vitamin A/0.5 mM GlutaMAX™/Growth Factors media.
12. Plate 0.2 ml/$cm^2$ of suitable substrate, such as the Ultra Low Attachment Plates (Corning Cat #3473).
13. Incubate cells at 37°C, 5% $CO_2$, 9% or 20% Oxygen incubator.
14. After 4-7 days in culture, neurospheres may be harvested by aspiration for differentiation or further expansion.


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**C-STEM CELL YIELD MAXIMIZATION**

Cell viability is maximized by incubating cells with papain, as follows:

15. Disperse neurospheres in each well using a 1 ml pipette.
16. Transfer floating cells and media into 15 ml centrifuge tubes. Refill well with 0.2 ml warm NeuroPrep Medium.
17. Centrifuge cells down for 1 minute at 1,100 rpm (200 x G).
18. Discard supernatant and flick the tube to disperse cell pellet.
19. Resuspend cells in 0.2 ml NPM and return them to the original well.
20. Incubate at 37°C, **ambient $CO_2$** for 10 minutes.
21. Disperse cells using a 1 ml pipette and examine under a microscope for single cells, for example:
    a. Add 40μl resuspended cells to 40μl 0.4% trypan blue;
    b. Observe cells and clusters under a hemacytometer;
    c. If greater than 10% of cells are in clusters, repeat steps 17-21.
22. Remove NPM by centrifuging cells down (1100 rpm, 1 min) and resuspending them in Neurobasal/B27/0.5 mM glutamine media to the desired concentration.
23. If needed, pass cells by replating at 3,000 cells/$mm^2$ on uncoated plastic substrate (see Step 11 for recommendation).
24. To differentiate cells, plate onto plastic substrate coated with 100 μg/ml poly-D-lysine.

**D-STEM CELL DIFFERENTIATION (OPTIONAL)**

25. For highly enriched neurons, plate cells in Neurobasal (Invitrogen Cat. #: 21103-049) + B27 Supplement (Invitrogen Cat. #: 12587-010) + 0.5 mM glutamine (Invitrogen Cat#: 25030-149) at 40-400 cells/$mm^2$.
26. For highly enriched astrocytes, plate cells in Neurobasal + 10% horse serum + 2mM glutamine at 100-300 cells/$mm^2$.

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