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N-BASE MEDIUM (1X)

CATALOG NUMBER: N014-B **QUANTITY:** 500 mL

LOT NUMBER: **CONCENTRATION:** 1X

DESCRIPTION: N-Base Medium is a defined serum-free neural basal medium optimized for isolation and expansion of rodent hippocampal neural stem cells (NSCs) and neurons.

FORMULATION Proprietary serum-free formulation. Does not contain L-Glutamine. Must be supplemented prior to use.

APPLICATIONS: Isolation, expansion and differentiation of hippocampal neural stem cells.

FORMAT: Liquid

STORAGE/HANDLING: Upon receipt, store N-Base medium at 2-8°C. Refer to lot expiration date on label.

PROTOCOL: ***Isolation and Expansion of Hippocampal Neural Stem Cells:***

Materials and Preparation:

- Cold PBS (Millipore Cat. No. BSS-1005-A)
- E16 mouse embryos or E18 rat embryos
- Culture dishes
- Accutase (Millipore Cat. No. SF006)
- Fibronectin (Millipore Cat. No. FC010)
- ESGRO freezing medium (Millipore Cat. No. SF005)

Prepare 500 mL Neural Stem Cell Isolation/Expansion Medium as follows:

Component	Amount
DMEM/F12 (Millipore Cat. No. DF-041-B) w/ L-glutamine	291 mL
N-Base medium (Millipore Cat. No. N014-B)	291 mL
N21 medium supplement (Millipore Cat. No. SCM081)	10 mL
Neuro2 medium supplement (Millipore Cat. No. SCM013)	2.5 mL
BSA (Invitrogen Cat. No. 15260-037)	150 µL
B-ME (Millipore Cat. No. ES-007-E)	5 mL
EGF (Millipore Cat. No. 01-107)	20 ng/mL
bFGF (Millipore Cat. No. GF003)	20 ng/mL

Prepare Fibronectin coated culture dishes: Dilute Fibronectin 50x in PBS to obtain a 1 mg/mL solution in PBS. Coat for 1 hour at 37°C. Use and remove Fibronectin solution before plating cells, or store at 4°C without removing Fibronecting. Do not let dry out.

1. Isolate whole embryos. Decapitate embryos and collect heads in cold PBS.
2. Remove skin and cut telencephalon into two hemispheres.

3. View the hemispheres from the medial lateral side and cut out hippocampus (thickened region lining the curved medial edge of the cortex). Dissect out hippocampus by making a longitudinal cut through the border and the cortex. Remove meninges surrounding the hippocampus.
4. Collect hippocampi in PBS in a conical 15 mL tube, and let them settle.
5. Remove PBS, add 2 mL Accutase and leave 2 min at RT.
6. Pipet up and down (3-5x) with a 1 mL Pipetman until a single cell suspension is obtained.
7. Add 2 mL of Neural Stem Cell Isolation/Expansion Medium (see previous page)
8. Spin down at 1000 rpm for 5 min.
9. Remove supernatant and add 2 mL of Neural Stem Cell Isolation/Expansion Medium.
10. Count cells and repeat step 8 and 9.
11. Freeze cells at 0.5 -1 x10^6 cells/mL or plate cells onto Fibronectin coated wells at 5x10^4 cells/cm^2. Refer to Figure 1 for morphology of hippocampal neural cells.

Differentiation of Hippocampal Neurons:

For differentiation, plate cells on Fibronectin coated wells at a concentration of 5x10^4 cells/cm^2. The next day, exchange the Neural Stem Cell Isolation/Expansion with medium without growth factors EGF and bFGF. Exchange medium without growth factors every 2-4 days. After 10-14 days overt differentiation occurs. Refer to Figure 2 for differentiated neurons.

REPRESENTATIVE LOT DATA:

Isolation of rodent hippocampal neural stem cells

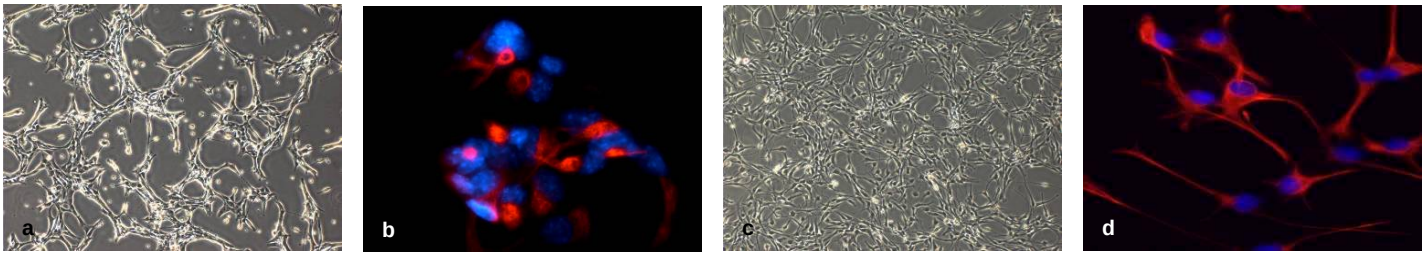

Fig 1. Isolation and expansion of rodent hippocampal neural stem cells to passage 3 (P3). **(a,b**) Mouse fetal hippocampal neural stem cells undifferentiated at P3 and **b)** stained with nestin antibody (red). **(c, d**) rat fetal hippocampal neural stem cells undifferentiated at P3 and **d)** stained with nestin antibody. Nuclei are visualized with blue DAPI staining.

Differentiation of rodent hippocampal neural stem cells

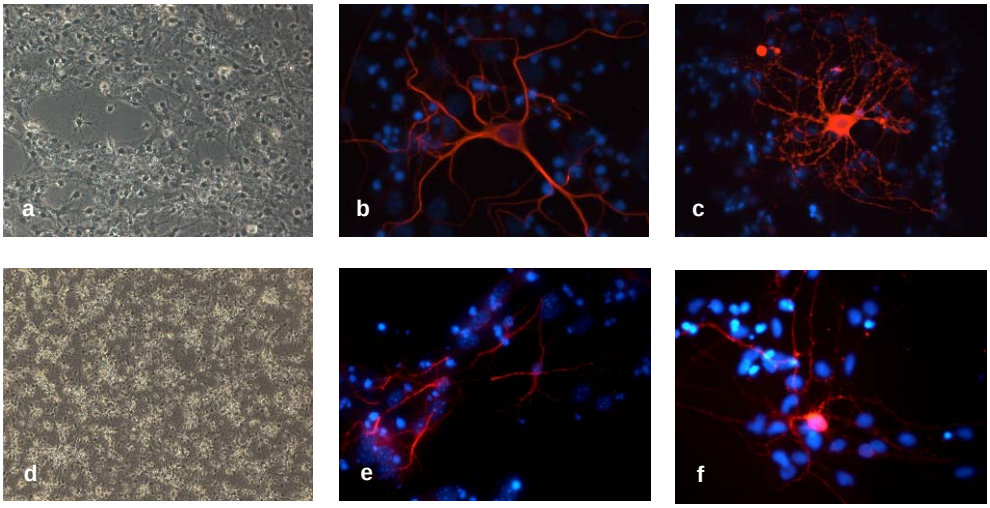

Fig 2. Differentiation of rodent hippocampal stem cells at passage 3 (P3). **(a – c)** Mouse fetal hippocampal neural stem cells differentiated at P3, **b**) stained with MAP-2 antibody, and **c**) stained with CamKII antibody. **(d - e)** rat fetal hippocampal neural stem cells differentiated at P3, **e**) stained with MAP-2 antibody, and **f**) stained with CamKII antibody. Antibody stainings are red, nuclei are visualized with blue DAPI staining.

RELATED PRODUCTS:

2-Mercaptoethanol (Millipore Cat. No. ES-007-E)
DMEM/F12, with L-glutamine (Millipore Cat. No. DF-041-B)
L-glutamine solution, 200mM (Millipore Cat. No. TMS-002-C)
N21 medium supplement (Millipore Cat. No. SCM081)
Neuro2 medium supplement (Millipore Cat. No. SCM012)
Recombinant bFGF (Millipore Cat. No. GF003)
Recombinant EGF (Millipore Cat. No. 01-107)

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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