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N21 MEDIUM SUPPLEMENT (50X)

CATALOG NUMBER: SCM081 **QUANTITY:** 10 mL

LOT NUMBER:

DESCRIPTION: N21 Medium Supplement is an optimized serum-free supplement developed for isolation and expansion of neurons from mouse and rat hippocampus. In addition to its use for long term neuronal survival, it has also been validated for serum-free culture of mouse ES cells and human ES cell derived neural progenitor cells.

APPLICATIONS:

- Isolation, expansion and differentiation of rodent hippocampal neural stem cells (Protocol A)
- Monolayer neuronal differentiation and Neural Stem Cell isolation from mESCs (Protocol B)
- Serum-free maintenance of a variety of cell lines.

FORMULATION: N21 Medium Supplement is a proprietary formulation that is supplied as an aqueous solution. Optimal working concentration of the medium supplement must be determined for specific uses and cell types. Typically, N21 supplement is diluted 1:50 for isolation and expansion of rodent hippocampal neurons.

QC PROTOCOL: Each lot of N21 supplement is tested in a clonal assay for mouse ES cell colonies, and for its ability to differentiate mouse ES cells to neurons.

STORAGE/HANDLING: Maintain at -20°C until expiration date. Protect from light. Once thawed, aliquot unused portion into smaller volumes and store at -20°C until future use. Once added to the cell culture medium, the product is stable at 4°C for 2 weeks or in the dark at -20°C for 2 months. Avoid repeat freeze and thaw cycles. N21 medium supplement can be thawed and re-frozen once.

PROTOCOL A Isolation, expansion and differentiation of rodent hippocampal neural stem cells:

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, with L-glutamine (Millipore Cat. No. DF-041-B)	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

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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):

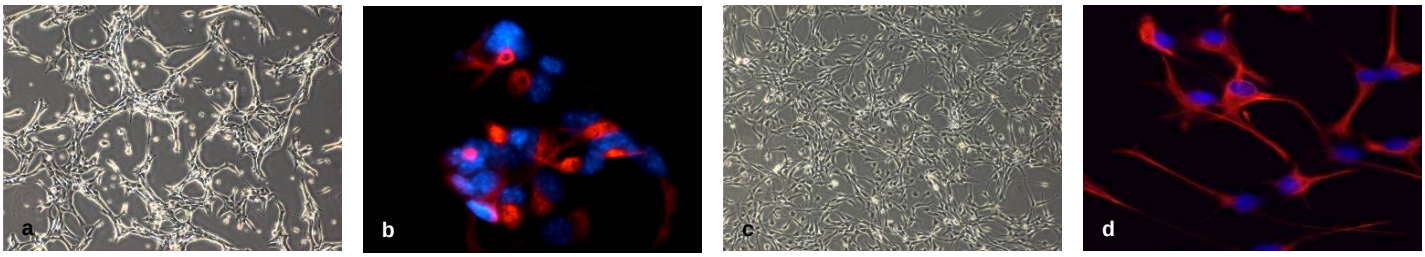

Fig 1. Isolation and expansion of rodent hippocampal neural stem cells to passage 3 (P3). (a,b) Mouse fetal hippocampal neural stem cells undifferentiated at P3 and b) stained with nestin antibody (red). (c, d) rat fetal hippocampal neural stem cells undifferentiated at P3 and d) stained with nestin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

Representative Lot Data (differentiation of rodent hippocampal neural stem cells):

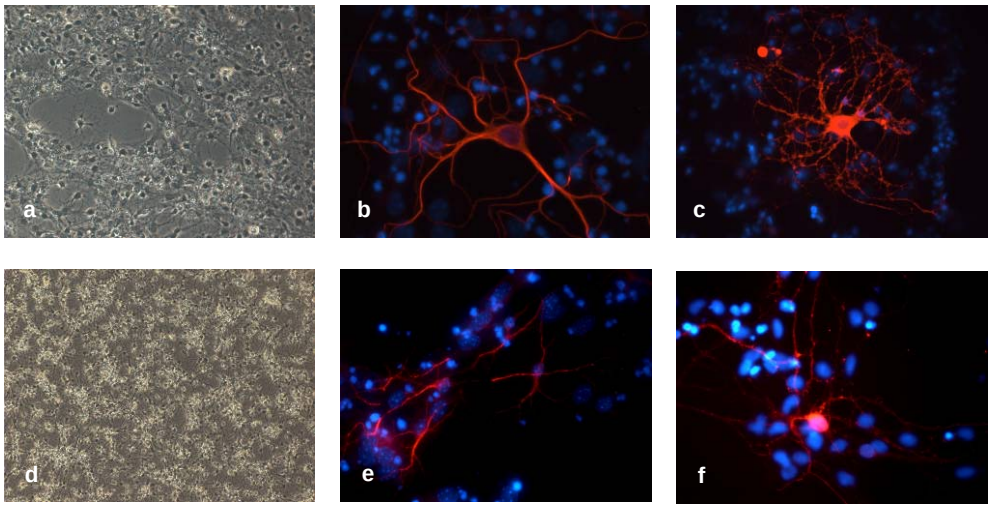

Fig 2. Differentiation of rodent hippocampal stem cells at passage 3 (P3).(a – c) Mouse fetal hippocampal neural stem cells differentiated at P3, b) stained with MAP-2 antibody, and c) stained with CamKII antibody. (d - e) rat fetal hippocampal neural stem cells differentiated at P3, e) stained with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

PROTOCOL B Monolayer neuronal differentiation and Neural Stem Cell isolation from mouse ES cells:

Monolayer Neuronal Differentiation

Materials and Preparation:

- 0.1% Gelatin
- Culture dishes (6 wells)
- ES2N basal medium (Millipore Cat. No. SCM083)
- N21 medium supplement (Millipore Cat. No. SCM081)
- Neuro2 medium supplement (Millipore Cat. No. SCM012)
- Mouse ES cells (Millipore Cat. Nos. CMTI-1, CMTI-2, SCR012)

Monolayer Neuronal Differentiation Medium: Add 5 mL of N21 supplement (Millipore Cat. No. SCM081) and 1.25 mL Neuro2 supplement (Millipore Cat. No. SCM012) to 250 mL ES2N Basal medium (Millipore Cat. No. SCM083).

Coat 6-well dishes with 0.1% gelatin 1- 4 hours at room temperature. *Note: Use fresh Gelatin. Do not coat for less than one hour or more than 4-6 hours.*

1. Passage serum-free and feeder-free mouse ES and iPS cells with Accutase, wash twice and count cells with a hemocytometer.
2. Plate 1-3 x 10^4 cells/cm^2 (1-3x 10^5 cells per 6-well plate). *Note: Plating the correct density is crucial. Each cell line needs optimization.*
3. Remove gelatin and add correct number of cells in 30 – 200 µL volume of monolayer neuronal differentiation medium (see above).

4. Add 3 - 4 mL of monolayer neuronal differentiation medium to each well and place at 37°C incubator with 5% CO_2.
5. Change medium every 1-2 days, gently aspirate undifferentiated cells. Depending on the cell line there can be cell death around day 3-5 associated with the assay.

The assay is completed on day 9-12, with up to 80-90% of neurons generated.

Representative Lot Data (monolayer neuronal differentiation):

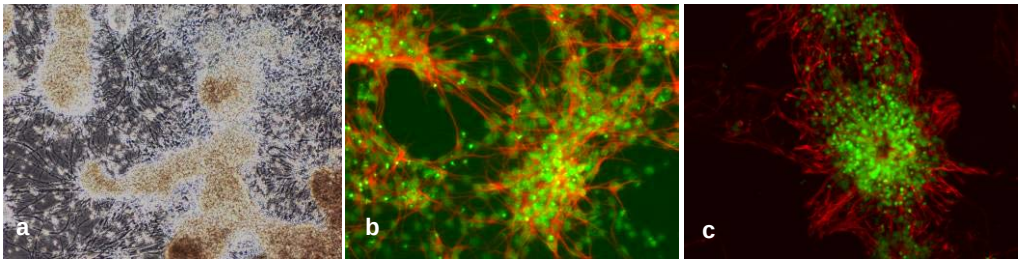

Fig 3. Monolayer neuronal differentiation with SCM083 containing N21. **a)** Bright field picture of Day 12 neuronal differentiation **b**) MAP-2 antibody staining. **c**) Nestin antibody staining. Antibody stainings are red, nuclei are visualized with green DAPI staining.

Neural Stem Cell Isolation from Mouse ES Cells:

Use feeder-free mouse ES cells with the monolayer neuronal differentiation medium and plate cells onto 0.1% Gelatin coated 6 wells. Feed cells with monolayer neuronal differentiation medium to allow for monolayer differentiaion. On day 5-7 neuronal rosettes appear. At this point neural stem cells can be isolated by replating cells in the Neural Stem Cell Isolation/Expansion medium containing bFGF and EGF and following the protocol for neural stem cell maintenance below.

1. Follow the protocol for the monolayer neuronal differentiation using the monolayer neuronal differentiation medium (see previous page).
2. On day 5 prepare Fibronectin coated dishes as described in Protocol A.

   Note: 0.1% Gelatin may be used for expansion of neural stem cells, however, attachment is weak.
3. On day 5-7 of the monolayer differentiation culture, wash cells with PBS and add just enough Accutase to cover all cells.
4. Incubate at RT for 3-5 min, and resuspend cells to obtain a single cell suspension.
5. Transfer cells into a conical tube with 2 mL of prewarmed Neural Stem Cell Isolation/Expansion Medium containing bFGF and EGF (see Protocol A).
6. Spin down at 1000 rpm for 5 min.
7. Remove supernatant and repeat wash (step 5,6).
8. Plate cells at $5x10^4$ cells/cm^2.

Representative Lot Data (isolation of neural stem cells from mouse ES cells):

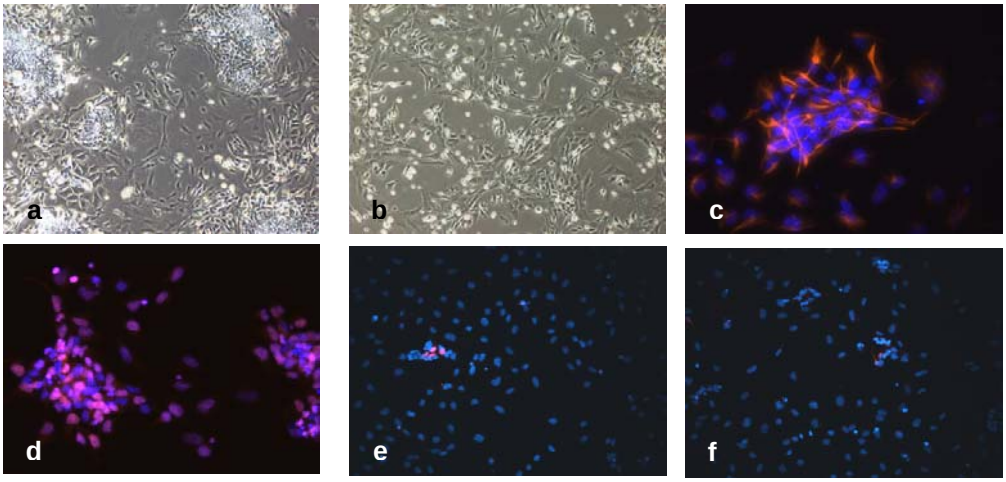

Fig 4.Isolation of neural stem cells from mESC's at Day 5. **a)** Neuronal rosette formation during monolayer differentiation of mESC's. **b)** re-plating of neuronal rosettes at day d 5. **c)** Nestin antibody staining. **d)** Sox-2 antibody staining overlaps with DAPI nuclear staining **e)** Oct4 antibody staining, note that although Oct 4 expression in most fields is absent, occasionally upon careful screening, some remaining Oct4 expressing cells can be found. **f)** Map-2 antibody staining is largely absent. Antibody stainings are red, nuclei are visualized with blue DAPI staining.

RELATED PRODUCTS:

Nestin monoclonal antibody (Millipore Cat. No. MAB353)

MAP-2 monoclonal antibody (Millipore Cat. No. MAB3418)

Sox-2 polyclonal antibody (Millipore Cat. No. AB5603)

Oct-4 monoclonal antibody (Millipore Cat. No. MAB4419)

DAPI (Millipore Cat. No. S7113)

CMTI-1 mouse ES cell line (Millipore Cat. No. SF-CMTI-1)

Alkaline Phosphatase Detection Kit (Millipore Cat. No. SCR004)

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