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1. Description

Components 500 mL MACS® Neuro Medium, w/o Glutamine

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the bottle label.

MACS Neuro Media are for research use only and not for diagnostic or therapeutic use.

1.1 Background information

MACS Neuro Medium has been optimized for the culture of neural cells after tissue dissociation using Neural Tissue Dissociation Kits (refer to 7. Related products) and subsequent cell isolation using magnetic cell sorting.

MACS Neuro Medium is a serum-free cell culture medium, which promotes optimum growth and long-term survival of cells from the mouse, rat, or human central or peripheral nervous system (CNS or PNS, respectively).^{1,2}

Supplemented with the appropriate growth factors and supplements, MACS Neuro Medium is suitable for the formation of neurospheres (neurosphere assay) from magnetically isolated neural progenitor/stem cells as well as for the culture of magnetically sorted neuronal and glial precursor or differentiated cells from various tissues of the developing, postnatal and adult nervous system.

MACS Supplement B27 PLUS (# 130-093-566) is recommended for low density plating, long-term viability, and growth of CNS and PNS neurons. MACS Supplement B27 PLUS is a chemically-defined, serum-free supplement based on published formulations of B27 with re-optimized formulations of added components.³

When added to MACS Neuro Medium, MACS Supplement B27 PLUS supports the growth of neuronal cells without an astrocyte feeder layer as well as the growth of neuronal tumor cell lines.

1.2 Reagent and instrument requirements

General reagents and plastic ware requirements

- (Optional) MACS Tissue Dissociation Products (refer to 7. Related products)
- (Optional) Trypan Blue Stain (Invitrogen™, # 15250-061) and a hemocytometer
- (Optional) Erythrosine red and a hemocytometer
- 24-well plate or cultivation flask
- (Optional) MACS Cell Separation Products (refer to 7. Related products)
- Pre-Separation Filters (# 130-041-407) to remove cell clumps
- MS Columns (# 130-042-201)
- VarioMACS™ Separator (# 130-090-282)
- OctoMACS™ Separator (# 130-042-109)
- MiniMACS™ Separator (# 130-042-102)

Lab equipment

- Laminar flow hood (biohazard containment hood)
- CO₂ incubator, 37 °C with 5% CO₂ in air and > 95% humidity

Reagents and materials for culture, staining, and characterization of neural cells

- MACS Neuro Medium (# 130-093-570)
- MACS Supplement B27 PLUS (# 130-093-566)
- Laminin (Sigma-Aldrich®, # L 2020)
- Accutase™ (Millipore, # SCR005)
- Poly-L-Lysine
- L-Glutamine
- Fetal bovine serum (FBS)
- Penicillin/Streptomycin (Pen/Strep)
- Recombinant human EGF, e.g., Human EGF (# 130-093-825)
- Recombinant mouse EGF, e.g., Mouse EGF (# 130-094-036)
- Recombinant human basic FGF, e.g., Human FGF-2 (# 130-093-839)

For a complete list of cytokines and growth factors refer to www.miltenyibiotec.com/cytokines.

- (Optional) N2-Supplement
- (Optional) G5-Supplement

2. Preparation of cells

2.1 Sample preparation and dissociation of neural tissues to single-cell suspensions

Neural tissues can be gently and efficiently dissociated to single-cell suspensions by use of the Neural Tissue Dissociation Kits, also in combination with the gentleMACS™ Dissociator (# 130-093-235). Neural tissues from embryonic, postnatal, and adult mouse or rat tissue sections or whole brain preparations can be processed. The dissociated cells are immediately ready for further *in vitro* or *in vivo* applications. Myelin Removal Beads (# 130-094-544) allow for the specific removal of myelin debris during sample preparation from single cell suspensions, leading to a higher purity and recovery of target cells, and significantly improved efficiency of cell separations and immunostainings.

For a list of MACS Products for the dissociation of neural tissues, please refer to 7. Related products. For the MACS neural tissue dissociation procedure, please refer to the respective data sheet.

2.2 Isolation of neural cells

Specific neural cell types can be isolated using magnetic cell sorting with specific monoclonal antibody conjugates within minutes. Specific cell-subtype and subset isolations of neural cell suspensions can be achieved, supporting rapid and efficient attainment of pure cell cultures.

For a list of MACS Products for the isolation of neural cells, please refer to 7. Related products. For MACS isolation procedures, please refer to the respective data sheet.

▲ For isolation of neural progenitor cells, please use Anti-Prominin-1 MicroBeads (# 130-092-333) and two MS Columns.

▲ For isolation of neuronal precursor cells, please use Anti-PSA-NCAM MicroBeads (# 130-092-966), Anti-PSA-NCAM antibodies (# 130-093-273, # 130-093-274), and two MS Columns.

▲ For isolation of glial precursor cells, please use Anti-A2B5 MicroBeads (# 130-093-388), Anti-A2B5 antibodies (# 130-093-581, # 130-093-582, # 130-093-393, # 130-093-394), and two MS Columns.

3. Preparation and culture of neural cells

▲ Perform all of the following steps under sterile conditions in a laminar flow hood.

3.1 Preparation of coated plates

We recommend coating tissue culture plastic- or glasswares with poly-L-Lysine for use of neural precursor cell culture. The following procedure is recommended:

1. Add 300 µL poly-L-Lysine to one well of a 24-well plate or add enough to cover the whole surface of a flask.
2. Incubate for 20 minutes at room temperature.
3. Wash the tissue cultureware three times with at least 500 µL (24-well plate) sterile phosphate-buffered saline (PBS).

3.2 Culture of neural cells

1. Pre-warm MACS Neuro Medium to 37 °C in a water bath or incubator, and supplement it as indicated in table 1.

▲ **Note:** (Optional) Add 1% Penicillin/Streptomycin to the medium to avoid bacterial contamination of the cell culture.

▲ **Note:** Growth factors and MACS Supplement B27 PLUS should always be added fresh to MACS Neuro Media.

2. Prepare a suspension of neural cells (refer to 2. Preparation of cells) in MACS Neuro Medium.
3. Cells are plated at the desired concentration in MACS Neuro Medium supplemented as indicated in table 1 (refer also to 6. Appendix).

▲ **Note:** (Optional) Use round coverlids coated with poly-L-Lysine for later fixation and immunolabeling of the cells.

4. Incubate the vessels at 37 °C in a humidified incubator with 5% CO₂ and >95%.
5. When culturing the cells for longer than 4 days, one-half of the medium is removed on day 2 and replaced with an equal volume of medium. Subsequent changes of medium should be made accordingly every 2 days thereafter.

Celltype	Prominin ⁺	PSA-NCAM ⁺	A2B5 ⁺
Medium	9560 µL	8600 µL	8800 µL
B27 Supplement (50×)	200 µL (1×)	200 µL (1×)	-
(Optional) Pen/Strep (100%)	100 µL (1%)	100 µL (1%)	100 µL (1%)
L-Glutamine (200 mM)	100 µL (2 mM)	100 µL (2 mM)	100 µL (2 mM)
FBS (100%)	-	1000 µL (10%)	1000 µL (10%)
EGF (10 µg/mL)	20 µL (20 ng/mL)	-	-
FGF-2 (10 µg/mL)	20 µL (20 ng/mL)	-	-

Table 1: Supplementation for 10 mL of MACS Neuro Medium for different neural cell types.

3.3 Characterization of neural cells

A) Prominin-1 positive neurospheres

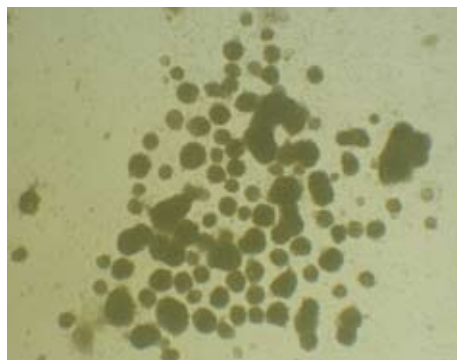


Figure 1:
Light microscopic picture of neurosphere formation after magnetic cell sorting using Anti-Prominin-1 MicroBeads after 7 days of cultivation in MACS Neuro Medium supplemented with MACS Supplement B27 PLUS. Cells were prepared from CD1 mouse brain (P3) using the Neural Tissue Dissociation Kit (P).

B) PSA-NCAM positive neuronal precursor cells

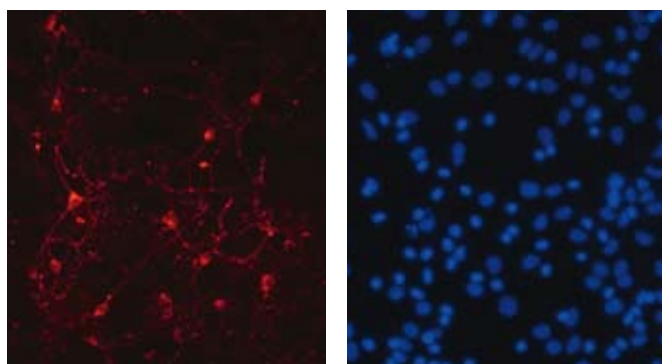


Figure 2:
Light microscopic pictures
Cells were prepared from CD1 mouse brain (P3) using the Neural Tissue Dissociation Kit (T).

Left panel: After 7 days of cultivation in MACS Neuro Medium supplemented with MACS Supplement B27 PLUS. PSA-NCAM positive cells were processed for immunofluorescent detection of anti-GAD67 antibody and goat anti mouse AF-594 (red), a protein marker for neuronal precursor cells.

Right panel: Nuclei are stained by DAPI (blue).

C) A2B5 positive glial precursor cells

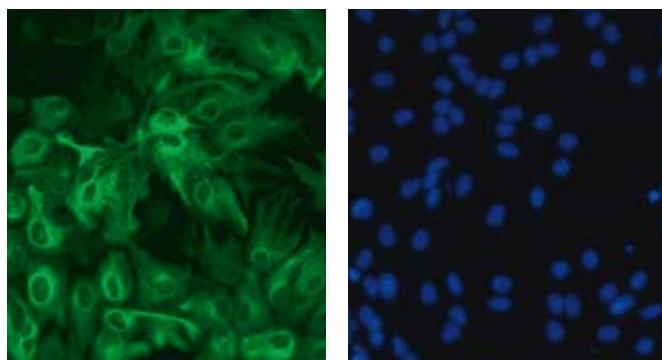


Figure 3:
Light microscopic pictures
Cells were prepared from CD1 mouse brain (P3) using the Neural Tissue Dissociation Kit (T).

Left panel: After 7 days of cultivation in MACS Neuro Medium supplemented with MACS Supplement B27 PLUS. A2B5 positive cells were processed for immunofluorescent detection of anti-GFAP antibody and goat anti rabbit AF-488 (green), a protein marker for glial precursor cells.

Right panel: Nuclei are stained by DAPI (blue).

4. Quality control testing

MACS Neuro Medium formulations undergo quality control testing for pH, osmolality, endotoxin, and absence of bacterial or fungal contamination. For growth promotion and absence of toxicity, the medium is supplemented with MACS Supplement B27 PLUS and tested in a growth assay utilizing a B104 (neuroblastoma) cell line.

5. References

- Brewer, G.J. *et al.* (1993) Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. *J. Neurosci. Res.* 35: 567–576.
- Brewer, G.J. (1997) Isolation and culture of adult rat hippocampal neurons. *J. Neurosci. Methods* 71: 143–155.

6. Appendix: Plate sizes for *in vitro* culture of neural cells

Cells should be resuspended according to total cell number in varying volumes of culture medium and plate size as indicated in table below.

Total cell number	Medium volume to add	Culture plate	Well area
0.5×10^5	0.15 mL	96 well	0.32 cm ²
1.50×10^5	0.50 mL	48 well	1.00 cm ²
3.00×10^5	1.00 mL	24 well	2.00 cm ²
6.00×10^5	2.00 mL	12 well	4.00 cm ²
14.40×10^5	5.00 mL	6 well	9.60 cm ²

7. Related products

Neural Tissue Dissociation Kit (P)	# 130-092-628
Neural Tissue Dissociation Kit (T)	# 130-093-231
Neural Tissue Dissociation Kit – Postnatal Neurons	# 130-094-802
gentleMACS™ Starting Kit	# 130-093-235
Myelin Removal Beads, human, mouse, rat	# 130-094-544
CD133 MicroBead Kit, human	# 130-050-801
Anti-Prominin-1 MicroBeads, mouse	# 130-092-333
Anti-A2B5 MicroBeads, human, mouse, rat	# 130-093-388
Anti-PSA-NCAM MicroBeads, human, mouse, rat	# 130-092-966
CD11b (Microglia) MicroBeads, human and mouse	# 130-093-634
CD90.2 MicroBeads, mouse	# 130-049-101
CD271 MicroBead Kit (PE), human	# 130-092-819
CD271 MicroBead Kit (APC), human	# 130-092-283
Human EGF	# 130-093-825
Mouse EGF	# 130-094-036
Human FGF-2	# 130-093-839

For more information about antibodies refer to www.miltenyibiotec.com.

All protocols and data sheets are available at www.miltenyibiotec.com.

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