GIBCO
invitrogen cell culture

NEUROBASAL™ and NEUROBASAL™-A Media

Serum-free basal medium for the long-term viability of hippocampal and other neurons of the CNS.

Product	Cat. No.	Pre-natal	Post-natal	with Phenol red	Tumor Cell lines
NEUROBASAL™ Medium	21103	X		X	
NEUROBASAL™ Medium without Phenol Red	12348	X			
NEUROBASAL™A Medium	10888		X	X	X
NEUROBASAL™A Medium without Phenol Red	12349		X		X

Instructions for use:

Prior to use, NEUROBASAL media must be supplemented with 0.5 mM L-glutamine for primary cells and 2.0 mM L-glutamine for neuronal phenotype tumor cells or neural stem cells. In addition, either a serum-free supplement or serum needs to be added. Recommended supplement is B-27 Supplement (Cat. No. 17504) for hippocampal and other CNS neurons. For embryonic neurons the addition of 25 uM glutamic acid is also recommended for the plating step. For neural stem cells N-2 Supplement (Cat. No. 17502) or B-27 Supplement without retinoic acid (Cat. No. 12587) is recommended. For tumor cell lines of glial origin G-5 (Cat. No. 17503) is recommended.

Protocols for isolation of primary hippocampal and cortical neurons can be found in the Tech-online portion of the Invitrogen website, www.invitrogen.com

Units of 500mL

Storage conditions: 2° to 8° C, in the dark

Intended Use

NEUROBASAL Cat. No. 21103 - when supplemented with B27 is intended to give optimal growth and long- term survival of rat embryonic hippocampal neurons[1], and growth and survival of neurons from embryonic rat striatum, substantia nigra, septum and cortex, and neonatal rat cerebellum and dentate gyrus[2].

NEUROBASAL Cat. No. 12348 - Specific use for receptor studies such as estrogenic receptors, downstream protein purification studies or other processes where the presence of Phenol Red is undesirable

NEUROBASAL -A Cat. No. 10888 - when supplemented with B27[2] and β-FGF is intended to maintain long-term growth and viability of rat postnatal and adult hippocampal neurons.

-The osmolality of NEUROBASAL -A was demonstrated to be optimal for postnatal and adult CNS neurons.

- NEUROBASAL when supplemented with N2[3], B27 or serum is effective for the growth of tumor cell lines of neuronal origin.

NEUROBASAL -A Cat. No. 12349 **-** Specific use for receptor studies such as estrogenic receptors, downstream protein purification studies or other processes where the presence of Phenol Red is undesirable

Background

NEUROBASAL with B27 (Cat. No. 17504) has shown excellent long-term viability of rat embryonic hippocampal neurons even after four (4) weeks in culture with greater than 90% viability for cells plated at 640/mm^2 and greater than 50% viability for cells plated at 160/mm^2. Glial cell growth at five (5) days is reduced to less than 0.5% for a nearly pure neuronal population[1].

The growth of adult CNS neurons requires gentle separation of their numerous connections, a density gradient for the separation of oligodendrocytes and enrichment of neurons, an adequate substrate for attachment and a dedicated medium for growth. Brewer[1] has shown that NEUROBASAL -A supplemented with B27 and adequate isolation methods permit the isolation of spherical remnants of hippocampal neurons from any age rat and promote the regeneration of axon and dendrite-like processes.

NEUROBASAL and NEUROBASAL -A must be supplemented with N2 or B27 and 0.5 mM L-glutamine. For the initial plating of embryonic primary hippocampal neurons, it is suggested that 25 µM (3.7 µg/mL) glutamate be added to the NEUROBASAL medium. Both media support the growth of nearly pure populations of neural cells without the need of an astrocyte feeder layer. Both media, when supplemented with B27, contain anti-oxidants to reduce reactive oxygen damage and they do not contain the excitatory amino acids, glutamate and aspartate, making it amenable to the study of these neurotransmitters.

Cell Plating:

a. Autoclaved glass coverslips (12 mm diameter Assistant brand from Carolina Biological) previously coated with 50 µg/mL poly-D-lysine in water (135 kD, Sigma) should be applied overnight, aspirated, rinsed once with water and allowed to dry one hour.
b. Cells are plated at the desired concentration (i.e. 90-320 cells/mm^2) in 60 - 150µL NEUROBASAL -A/B27(postnatal) or NEUROBASAL /B27(prenatal).
c. One hour after plating and incubation (ambient oxygen with 5 % CO_2 is acceptable but 9 % oxygen with 5 % CO_2 is preferable), the coverslip is quickly picked up, allowed to drain and transferred to 0.4 mL NEUROBASAL -A/B27 in a 24-well plate at 37^0 C. For postnatal neurons, the medium is aspirated, the coverslips rinsed once with warm NEUROBASAL -A and the cells refed with NEUROBASAL -A supplemented with B27, 0.5 mM L-glutamine, 1% penicillin-streptomycin (Cat. No. 15070) and 5 ng/mL β-FGF (Cat. No. 13256).
d. When culturing the cells for longer than 4 days, one-half of the medium is removed on day 3 or 4 and replaced with an equal volume of medium now containing 10 ng/mL β-FGF (postnatal neurons). Subsequent medium changes for prenatal neurons (4 days) should be made with NEUROBASAL without glutamate, to reduce glutamate toxicity in the culture. With neuroblastomas, the glutamate should be included in the medium for both plating and subsequent media changes.

Improved long-term survival of hippocampal neurons may be obtained by the addition of 2-mercaptoethanol (Cat. No. 21985) at 25 µM[4].

Quality Control Testing

NEUROBASAL - formulations are tested for pH, osmolality, endotoxin and the absence of bacterial and fungal contamination. For growth promotion and absence of toxicity, the medium is supplemented with B27 and tested in a growth assay utilizing a B104 (neuroblastoma) cell line

References

[1] Brewer, G.J. Isolation and culture of adult rat hippocampal neurons. *J Neurosci. Methods*: in press 71: 145-158 (1997).

[2] Brewer, G.J., Torricelli, J.R., Evege, E.K. and Price,P.J. Optimized survival of hippocampal neurons in B27 supplemented NEUROBASAL™. A new serum-free medium combination. *J. Neurosci. Res*. **35**: 567-576 (1993).
Brewer, G.J. Serum-free B27/Neurobasal medium supports differential growth of neurons from the striatum, substantia nigra, septum, cerebral cortex, cerebellum, and dentate gyrus. *J. Neurosci. Res.* **42**, 674-683 (1995).
Price, P.J. and Brewer, G.J. Serum-free mediua for neural cell culture. Protocols for Neural Cell Culture, 3rd edition. Chapter19. Eds. S. Federaff and A. Richardson, Humana Press 255-264, (2001).

[3] Bottenstein, J.E. Defined Media For Dissociated Neural Cultures. *In: Current Methods In Cellular Neurobiology* **4**:107-130 (J.L. Barker, ed.), John Wiley and Sons (1983).

[4] Grill, R.J., Jr., Pixley, S.K. 2-Mercaptoethanol Is A Survival Factor For Olfactory, Cortical and Hippocampal Neurons In Short-term Dissociated Cell Culture. *Brain Res.* **613**:168-172 (1993).Ishii, K., Katayama, M., Hori, K., Yodoi, J., Nakanishi, T. Effects of 2- Mercaptoethanol on Survival and Differentiation of Fetal Mouse Brain Neurons Cultured In Vitro. *Neurosci. Letters* **163:** 159-162 (1993).

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You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

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