GIBCO™
Invitrogen Corporation

B-27 Supplement 50X

CAUTION: **Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.**

Cat. No. 17504 10 mL

Intended Use

B-27 Supplement is a 50X serum-free supplement designed for the long-term viability of hippocampal and other neurons of the central nervous system (CNS). It is intended for laboratory research use only.

Storage Conditions: -5 to -20°C, protect from light.

Features and Benefits

- B-27 when used as a supplement to NEUROBASAL™ (Cat. No. 21103) has been demonstrated to give optimal growth and long-term survival of rat embryonic hippocampal neurons, and growth and survival of neurons from embryonic rat striatum, substantia nigra, septum and cortex, and neonatal rat cerebellum and dentate gyrus[1].
- B-27 when used as a supplement to NEUROBASAL is effective for the growth of tumor cell lines of neuronal origin.
- B-27 when used as a supplement to NEUROBASAL-A (Cat. No. 10888) has been demonstrated to allow for the growth of postnatal and adult rat hippocampal and cortical neurons after further supplementation with β-FGF (Cat. No. 13256)[2].
- B-27 Supplement has been demonstrated to allow the expansion of EGF-responsive precursor cells from embryonic rat striatum and mesencephalon[3].
- B-27 when used as a supplement to NEUROBASAL supports the growth of nearly pure populations of neural cells without the need of an astrocyte feeder layer.
- B-27 Supplement contains a cocktail of antioxidants to reduce reactive oxygen damage.
- B-27 has a one year shelf-life when stored between -5 to -20°C.

Background

B-27 is an optimized serum substitute developed for low density plating and long-term viability and growth of hippocampal and other CNS neurons. By supplementing NEUROBASAL with B-27 and 0.5 mM L-glutamine Cat. No. 25030); excellent long-term viability of rat embryonic hippocampal neurons has been achieved even after four 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 is reduced to less than 0.5% for a nearly pure neuronal population[1].

When using B-27 as a supplement to NEUROBASAL it is suggested that 25 µM (3.7 µg/mL) L-glutamic acid be added to the medium for the initial plating of primary hippocampal neurons. Subsequent medium changes after day 4 should be made without glutamate. 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[4,5].

Application

In addition to low density growth of fetal hippocampal neurons, the combination of B-27 and NEUROBASAL has been shown to support the growth of neurons from embryonic rat striatium, substantia nigra, septum, cortex, and neonatal dentate gyrus and cerebellum[1].

The combination of B-27 Supplement with NEUROBASAL-A has been demonstrated to support the growth of postnatal and adult rat hippocampal and cortical neurons[2].

The combination of B-27 Supplement with a DMEM:F12 mixture has been demonstrated to support the expansion of EGF-responsive precursor cells from rat embryonic striatum and mesencephalon[3].

Quality Control Testing

B-27 Supplement is tested in a growth assay utilizing primary rat (Sprague Dawley) embryonic hippocampal neurons, 18 day gestation. Additional standard evaluations are for the absence of bacterial and fungal contaminants. B-27 is also tested for endotoxin at a 1X concentration.

Instructions for Use

As the B-27 Supplement is supplied as a 50X concentrate, you should add 2.0 mL to 100 mL of NEUROBASAL.

The following procedures have been found effective with 18-day gestation rat hippocampi and with neuroblastoma cell lines.

1. Coat culture vessels with a 0.05 mg/mL solution of cold poly-D-lysine (MW 30,000 - 70,000) and incubate for 1 hour or overnight. For primary cultures use 0.15 mL/cm^2 surface area. When using neuroblastoma cell lines coat the dish with 0.04 mL/cm^2 of poly-D-lysine. Poly-D-lysine solutions are stored at -20°C in polycarbonate tubes. Poly-D-lysine should be prescreened for toxicity.
2. Wash vessels with sterile, deionized cell culture grade water. Note: Vessels can now be used or stored for up to 2 weeks at 4° to 10°C in sterile deionized, distilled water. If vessels are to be stored, remove water about 1 hour prior to use.
3. To NEUROBASAL medium, add 0.5 mM L-glutamine, 25 µM L-glutamic acid, and 2% B-27 Supplement.
4. For primary hippocampal neurons (i.e. from Sprague Dawley rats at 18 days gestation) and other fetal neurons.

a. Embryos are recovered by C-section under nembutal anesthetic and desired region dissected.
b. Individual cells are isolated by trituration 10 times in 1 mL Hanks' Balanced Salt Solution w/o Ca^{++} and Mg^{++} (Cat. No. 14175) and supplemented with 1.0 mM sodium pyruvate (Cat. No. 11360) and 10 mM HEPES (Cat. No. 15630), pH 7.4 using a 9 inch siliconized Pasteur pipette with the tip barely fire polished.
c. Divalent cations are restored by dilution with 2 volumes HBSS (Cat. No. 14025) supplemented as above.
d. After allowing non-dispersed tissues to settle for 3 min., the supernatant is transferred to a 15 mL tube and centrifuged for 1 min. at 200 g.
e. The pellet is gently resuspended in 1 mL HBSS per brain and an aliquot taken for counting.
f. Cells are added to the wells with supplemented NEUROBASAL at 160/mm^2 or other desired concentrations.
g. Cultures maintained longer than 4 days should have one-half of the medium changed to B-27/ NEUROBASAL without L-glutamic acid on day 4 and then once per week. If the initial culture density is higher than 640 cell/mm^2, the medium should be changed twice a week.

1. **Cell Lines**

a. Some cell lines may require an initial attachment in 2% serum-supplemented NEUROBASAL medium. Serum-free NEUROBASAL supplemented with B-27 can then be added after incubation for 2 hours or overnight.
b. To transfer cells:
Remove spent media and wash cells with HBSS (Cat. No. 14175). Detach cells from the substratum with 0.25 % trypsin/1.0 mM EDTA Cat. No. 25300). Aspirate excess trypsin/EDTA solution. A strong tap to the vessel after 2-4 minutes should remove cells. Dilute cells in HBSS (Cat. No. 14025) containing 0.05% soybean trypsin inhibitor (Cat. No. 17075). Centrifuge at 1000X g for 2 min. at room temperature. Resuspend pellet in the plating medium at the desired plating concentration.

References:

1. Brewer, G.J., Torricelli, J.R., Evege, E.K., Price, P.J. Optimized Survival of Hippocampal Neurons in B-27 Supplemented NEUROBASAL™. A New Serum-free Medium Combination. *J. Neurosci. Res.* 35:567-576 (1993).
2. Brewer, G.J. Isolation and Culture of Adult Rat Hippocampal Neurons. *J. Neurosci. Methods* 71: 145-158 (1997).
3. Svendsen, C.N., Fawcett, J.W., Bentlage, C., Dunnett, S.B. Increased Survival of Rat EGF-Generated CNS Precursor Cells using B-27 Supplemented Medium *Exp. Brain Res.* 102: 407-414 (1995).
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).
5. 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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United States TECH-LINE℠ : 1 800 955 6288
Canada TECH-LINE: 1 800 757 8257

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

For research use only.
CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

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