GIBCO™
Invitrogen Corporation

B-27 Supplement Minus AO 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. 10889 10 mL

Intended Use

B-27 Supplement Minus AO is a 50X serum-free supplement designed for the study of aging, oxidative stress or toxicity, apoptosis and other studies of oxidative damage and rescue. It is intended for laboratory research use only.

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

Features and Benefits of B-27 Supplement Minus AO 50X (Cat. No. 10889)

B-27 Minus AO is identical to B-27 Supplement (Cat. No. 17504)[1] except for the removal of antioxidant components, vitamin E, vitamin E acetate, superoxide dismutase, catalase, and glutathione from the formula. Once antioxidants are added back product effects can be compared to a control complete B-27 Supplement 50X (Cat. No. 17504).

Features and Benefits of B-27 Supplement 50X (Cat. No. 17504)

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.

Both B-27 products have a one year shelf-life when stored between -5 to -20°C.

Background for B-27 Supplement 50X (Cat. No. 17504)

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 (Cat. No. 11048) be added to the medium for the initial plating of primary hippocampal neurons. Subsequent medium changes after day 4 should be made without L-glutamic acid. With neuroblastomas, the L-glutamic acid 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

B-27 Minus AO without its cocktail of antioxidants has been made available as a catalog item for the convenience of researchers interested in the role of free-radicals in aging, toxicity, apoptosis and chronic neurologic diseases.

As B-27 Minus AO is supplied as a 50X concentrate, 2.0 mL is added to 100 mL of NEUROBASAL.

Quality Control Testing

B-27 Minus AO is tested for endotoxin at a 1X concentration. It is also tested for the absence of bacterial and fungal contaminants.

Instructions for Use for B-27 Supplement 50X (Cat. No. 17504)

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°C 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 once per week. If the initial culture density is higher than 640 cell/mm^2, the medium should be changed twice a week.
5. 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).

For further information on this or other GIBCO™ products, contact Technical Services at the following:

United States TECH-LINE℠: 1 800 955 6288
Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

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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