Datasheet

Neuropan 27 Supplement

Defined Serum Substitute for Serum-free Culture of Neuronal Cells from the Central Nervous System

Product	Description	Catalogue-No.	Size
Neuropan 27 Supplement 20x	**Defined serum substitute for neuronal cells**	**P07-07010 P07-07100**	**10 ml 100 ml**
Neuropan 27 Supplement 50x	**Defined serum substitute for neuronal cells**	**P07-07210 P07-07200**	**10 ml 100 ml**

Product description
Neuropan 27 Supplement is provided as a 20x or 50x serum substitute designed for the long-term growth and survival of hippocampal and other neuronal cells of the Central Nervous System. Neuropan 27 is an optimized serum substitute developed for low density plating and long-term growth and survival of hippocampal and other neurons from the central nervous system. By supplementing Neuropan basal medium (P04-00900) with Neuropan 27 Supplement and 0.5 mM L-glutamine (Cat. No. P04-80100) excellent long-term viability of rat embryonic hippocampal neurons has been achieved after several weeks in culture. Glial cell growth is reduced to less than 0.1% for a largely pure neuronal cell culture.
When using Neuropan 27 as a supplement to Neuropan basal medium we suggest to add 25 µM (3.7 µg/ml) L-glutamate to the medium for initial plating of primary hippocampal neurons. Medium changes after day 4 should be done without glutamate. When culturing neuroblastomas, glutamate should be included for both plating and subsequent culturing of cells. Improved long-term survival of hippocampal neurons may be obtained by the addition of beta-mercaptoethanol (Cat. No. P07-05020) at 15-30 µM.

Storage conditions
Storage: -20°C in the dark
Stability: 1 year
Size: 10 ml, 100 ml, other sizes on request

Composition
Neuropan 27 Supplement is a serum substitute of defined composition containing albumin, insulin, transferrin, lipids and hormones. It is used as a supplement to Neuropan basal medium to support growth and survival of neuronal cells from the central nervous system.

Suitability
Neuropan 27 when used as a supplement to Neuropan basal medium (Cat. No. P04-00900) 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. The combination of Neuropan basal medium with Neuropan 27 Supplement is also effective for the growth of tumor cell lines of neuronal origin. On additional supplementation with FGF-2 (Cat. No. CB-1102024) a combination of Neuropan basal medium with Neuropan 27 Supplement has been shown to support the growth of postnatal and adult rat hippocampal and cortical neurons. Neuropan 27 Supplement has been demonstrated to allow the expansion of EGF-responsive precursor cells from embryonic rat striatum and mesencephalon. Neuropan 27 Supplement in conjunction with Neuropan basal medium supports the growth of nearly pure populations of neural cells without the need of an astrocyte feeder layer. Neuropan 27 contains a cocktail of antioxidants to reduce reactive oxygen damage. Neuropan 27 Supplement has a shelf-life of 1 year when stored at -20°C.

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Special Advantages

In addition to low density growth of fetal hippocampal neurons, the combination of Neuropan 27 and Neuropan basal medium has been shown to support the growth of neurons from embryonic rat striatium, substantia nigra, septum, cortex, and neonatal dentate gyrus and cerebellum. The combination of Neuropan 27 Supplement with Neuropan basal medium has been demonstrated to support the growth of postnatal and adult rat hippocampal and cortical neurons. In addition, a combination of Neuropan 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.

Instructions for Use

Neuropan 27 Supplement is supplied as a 20x or 50x concentrate; with 20x concentrate add 5.0 ml for 100 ml Neuropan basal medium and with 50x add 2.0 ml to 100 ml.

The following procedures are suggested with d18 gestation rat hippocampi and neuroblastoma cell lines:

- Coat culture vessels with a 0.05 mg/ml of cold poly-D-lysine (MW 30 – 70 kD) and incubate for 1 h or overnight. For primary cultures use 0.15 ml/cm^2 surface area. For 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.
- Wash vessel with sterile, de-ionized, cell culture grade water. Note: Vessels may be used immediately or stored for up to 2 weeks at 2-8°C in sterile water. If vessels are stored, remove water about 1 h before use.
- To Neuropan basal medium, add 0.5 mM L-glutamine, 25 µM L-glutamate, and Neuropan 27 Supplement.
- For primary hippocampal neurons (i.e. from rats at 18 days gestation) and other fetal neurons.
  - Embryos are recovered by caesarean section under anesthetic surgery and desired the region is dissected.
  - Individual cells are isolated by trituration 10x in 1 ml of HBSS w/o Ca^{2+}/Mg^{2+} (Cat. No. P04-33100) and supplemented with 1 mM sodium pyruvate (Cat. No. P04-43100) and 10 mM HEPES (Cat. No. P05-01100), pH 7.5 using a siliconized Pasteur pipette with the tip barely fire polished.
  - Divalent cations are restored by dilution with 2 volumes HBSS, w: Ca^{2+}/Mg^{2+} (Cat. No. P04-32100) supplemented as above.
  - After allowing non-dispersed tissue to settle for 3 min, the supernatant is transferred to a 15 ml tube and centrifuged for 1 min at 200g.
  - The pellet is gently re-suspended in 1 ml HBSS per brain and an aliquot is taken for counting.
  - Cells are added to the wells with supplemented Neuropan basal medium at 150/mm^2 or desired concentration.
  - Cultures maintained for more than 4 days should have 50% of the medium changed to Neuropan basal medium + Neuropan 27 Supplement without L-glutamate on day 4 and then about once a week. If the initial culture density is exceeding more than about 500 cells/mm^2, change medium twice a week.

Cell Lines

- Some cell lines may require an initial attachment in 1-3% serum-supplemented Neuropan basal medium. Serum-free Neuropan basal medium supplemented with Neuropan 27 can be added after an incubation for >2 h or overnight.
- Cell transfer: Remove spent medium and wash cells with HBSS (Cat. No. P04-33100). Detach cells from the plates with 0.25% trypsin/1.0 mM EDTA (Cat. No. P10-029100). Aspirate excess trypsin solution. Remove cells with a strong tap to the vessel after about 2 to 4 min. Dilute cells in HBSS containing 0.05% trypsin inhibitor (Cat. No. P10-033100). Centrifuge at 1000g for 2 min at room temperature. Re-suspend pellet in plating medium at the desired concentration and incubate at 37°C and 5% CO_2.

Technical support

For technical support, questions or remarks please contact your local PAN-Biotech partner or the technical department of PAN-Biotech via email (info@pan-biotech.com) or phone +49-8543-601630.

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