gibco by life technologies™

Neurobasal® and Neurobasal®-A Media

Description

Neurobasal® Medium and Neurobasal®-A Medium are basal media that, when supplemented with B-27® Supplement, meet the special cell culture requirements of pre-natal/embryonic and post-natal/adult brain neuronal cells, respectively. Both Neurobasal® and Neurobasal®-A Media can be used to cultivate neuronal cells from hippocampus, cortex and other regions of the brain. Neurobasal® and Neurobasal®-A Media when supplemented with B-27® Supplement have demonstrated optimal viability for both long and short term maintenance of homogeneous populations (<0.5% Glial cells) of neuronal cells without the need of an astrocyte feeder layer. Component deficient (Phenol Red Minus) Neurobasal® and Neurobasal®-A Medium are provided for receptor studies such as estrogenic receptors, downstream protein purification studies or other processes where the presence of phenol red is undesirable.

Product	Catalog no.	Amount	Storage	Shelf life*
Neurobasal® Medium	21103-049	500 mL	2°C to 8°C; Protect from light	12 months
Neurobasal® Medium Minus Phenol Red	12348-017	500 mL	2°C to 8°C; Protect from light	12 months
Neurobasal®-A Medium	10888-022	500 mL	2°C to 8°C; Protect from light	12 months
Neurobasal®-A Medium Minus Phenol Red	12349-015	500 mL	2°C to 8°C; Protect from light	12 months

* Shelf life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

- Neurobasal® Medium or Neurobasal®-A Medium, when supplemented with B-27® Supplement, contain anti-oxidants to reduce reactive oxygen damage and they do not contain the excitatory amino acids, glutamate and aspartate, making them amenable to the study of these neurotransmitters.
- Neurobasal®-A Medium, when supplemented with B-27® Supplement, is effective for the growth of tumor cell lines of neuronal origin.

Safety information

For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare media

1. To 100 mL Neurobasal® or Neurobasal®-A Medium, aseptically add 2 mL B-27® Supplement or other B-27® Supplement variants and 0.5 mM L-glutamine or GlutaMAX™-I.

   or

   To 100 mL Neurobasal® or Neurobasal®-A Medium, aseptically add 1 mL N-2 Supplement (100X) and 0.5–2 mM L-glutamine or GlutaMAX™-I.

   or

   To 100 mL Neurobasal® or Neurobasal®-A Medium, aseptically add 1 mL G-5 Supplement (100X) and 0.5–2 mM L-glutamine or GlutaMAX™-I.
2. Prior to initial plating of primary neurons further supplement Neurobasal® Medium with 25 µM (3.7 µg/mL) L-glutamic acid. Some cell lines may require an initial attachment in 2% serum-supplemented complete Neurobasal® Medium.
3. Improved long-term survival of hippocampal neurons may be obtained by the addition of 25 µM 2-mercaptoethanol.

Once supplemented, the complete Neurobasal medium is stable for up to one week when stored in the dark at 2°C to 8°C.

Culture conditions

Media: Complete Neurobasal® or Neurobasal®-A Medium

Culture type: Adherent

Culture vessels: Multiwell plate or T-flasks

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Neuronal cell culture

Primary neuronal cultures are indispensable in the field of neurobiology and pharmacology. Many researchers favor freshly isolated neuronal cells as they maintain their functional viability, but for convenience, Primary Rat Neurons are a flexible, ready-to-use and quality alternative to freshly isolated neurons (see **Recovery**).

Primary neuronal cells should be isolated following user optimized protocols to ensure isolation of desired cell type.

Plate cells for culture

1. Coat sterile glass coverslips (12-mm diameter) or other desired culture vessels with cold 0.05 mg/mL poly-D-lysine solution. For primary neurons, use 0.15 mL/cm^2 surface area and incubate for 1 hour at ambient temperature.
2. Remove the poly-D-lysine solution, and rinse twice with sterile water. (Rinse thoroughly, since poly-D-lysine can be toxic to the cells).
3. Leave the plates uncovered in the hood until the wells are completely dry. Plates can be used immediately once dry or can be stored dry at 4°C for up to 2 weeks.
4. Plate cells at the desired concentration (i.e., 90–320 cells/mm^2) in 60–150 µL Neurobasal®-A/B-27® (postnatal) or Neurobasal®/B-27® (prenatal).
5. Incubate for one hour (humidified atmosphere of 5% CO_2 in air is acceptable, 9% oxygen with 5% CO_2 is preferable) at 37°C.
6. Remove medium by tipping coverslip and touching edge to sterile dry surface.
7. Transfer coverslip to 24-well plate, with 0.4 mL prewarmed Neurobasal®-A/B-27® (postnatal) or Neurobasal®/B-27® (prenatal) per well.

8. For postnatal neurons, aspirate the medium, refeed cells with prewarmed Neurobasal®-A/B-27® further supplemented with 1% penicillin-streptomycin and 5 ng/mL basic-Fibroblast Growth Factor (bFGF).
9. Refeed cells (day 3 or 4 post-plating and every 3 days thereafter) by removing one-half of the medium and replacing with an equal volume. Medium changes for prenatal neurons should be made with Neurobasal®/B-27® without glutamate, to reduce glutamate toxicity in the culture. For postnatal neurons use Neurobasal®-A/B-27®, without glutamate, supplemented with 10 ng/mL bFGF.

   Note: Include glutamate in the medium for plating and subsequent media changes when culturing neuroblastoma cells.

Recovery

Primary neuronal cells are extremely fragile upon recovery from cryopreservation; **do not** centrifuge cells. Primary neuronal cells will adhere to bare plastic and glassware; to maximize cell recovery and yield we recommend pre-rinsing all plastic and glassware with complete Neurobasal®/B-27® medium before use.

1. Prepare poly-D-lysine coated sterile culture vessels ahead of time (see **Plate cells for culture** steps 1–3).
2. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath. Remove vial from water bath just before the last trace of ice has melted.
3. Rinse a pipette tip with complete medium and very gently transfer the cells from the cryovial to a prerinsed 15-mL conical tube.
4. Rinse the cryovial with 1 mL of pre-warmed complete Neurobasal®/B-27® medium, and transfer the rinse to the 15-mL tube containing the cells at a rate of one drop per second. Mix by gentle swirling after each drop.
5. Dropwise add 2 mL of complete Neurobasal®/B-27® medium to the tube (for a total suspension volume of 4 mL). Mix by gentle swirling after each drop.
6. Determine viable cell density using a Countess® Automated Cell Counter.
7. Plate ~1 × 10^5 cells per well in poly-D-lysine coated 48-well plate or an 8-chambered slide. Bring the cell suspension volume to 500 µL per well by adding complete Neurobasal®/B-27® medium.
8. Incubate the cells at 37°C in a humidified atmosphere of 5% CO_2 in air (9% oxygen with 5% CO_2 is preferable).
9. Feed the cells every third day by aspirating half of the medium from each well and replacing it with fresh complete medium without glutamate.

Related products

Product	Catalog no.
B-27® Supplement (50X), liquid	17504
B-27® Supplement Minus AO (50X), liquid	10889
B-27® Supplement Minus Vitamin A (50X), liquid	12587
G-5 Supplement (100X), liquid	17503
N-2 Supplement (100X), liquid	17502
GlutaMAX™-I, (100X), liquid	35050
L-Glutamine 200mM (100X), liquid	25030
Primary Rat Cortex Neurons	A10840
Primary Rat Hippocampus Neurons	A10841
Penicillin-Streptomycin, liquid	15070
FGF-basic Recombinant Human	13256
2-mercaptoethanol (1000X), liquid	21985
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190
CELLStart™ CTS™	A10142

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Use By:	Manufacturer	Batch code	Keep away from light	Temperature Limitation
				
Catalog number	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques	

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Important licensing information

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**