Neurobasal™ Medium and Neurobasal™-A Medium

Catalog Numbers 21103049, 10888022, 12348017, and 12349015

Pub. No. MAN0007306 **Rev.** 2.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product 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™ Medium and Neurobasal™-A Medium can be used to cultivate neuronal cells from hippocampus, cortex and other regions of the brain. Both 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 for an astrocyte feeder layer. Neurobasal™ Medium Minus Phenol Red and Neurobasal™-A Medium Minus Phenol Red are provided for receptor studies such as estrogenic receptors, downstream protein purification studies or other processes where the presence of phenol red is undesirable.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
Neurobasal™ Medium	21103049	500 mL	2–8°C; Protect from light	12 months
Neurobasal™ Medium Minus Phenol Red	12348017	500 mL		
Neurobasal™-A Medium	10888022	500 mL		
Neurobasal™-A Medium Minus Phenol Red	12349015	500 mL		

[1] Shelf life duration is determined from Date of Manufacture.

Procedural guidelines

- 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.

Culture conditions

Media: Complete Neurobasal™ Medium 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.

Prepare complete media

1. Aseptically add supplements to 100 mL Neurobasal™ Medium or Neurobasal™-A Medium according to one of the following conditions.
   - Add 2 mL B-27™ Supplement or other B-27™ Supplement variants and 0.5 mM L-glutamine or GlutaMAX™ Supplement.
   - Add 1 mL N-2 Supplement (100X) and 0.5–2 mM L-glutamine or GlutaMAX™ Supplement.
   - Add 1 mL G-5 Supplement (100X) and 0.5–2 mM L-glutamine or GlutaMAX™ Supplement.
2. Prior to initial plating of primary hippocampal neurons, further supplement Neurobasal™ Medium with 25 µM (3.7 µg/mL) glutamate.

   Some cell lines may require an initial attachment in 2% serum-supplemented complete Neurobasal™ Medium.

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

Cell culture procedure

Coat culture plates with Poly-D-Lysine

1. Dilute the Poly-D-Lysine solution in sterile DPBS to prepare a 50 µg/mL working solution.
2. Coat the surface of the culture vessel (German glass or cell culture grade plastic) with 50 µg/mL Poly-D-Lysine solution.

   For primary neurons, use 0.15 mL/cm^2 surface area.
3. Incubate the vessel for 1 hour at room temperature.
4. Remove the Poly-D-Lysine solution, and rinse the culture surface 3 times with sterile distilled water.

   Make sure to rinse the culture vessel thoroughly as excess Poly-D-Lysine solution can be toxic to the cells.
5. Remove distilled water and leave the coated culture vessel uncovered in the laminar hood to dry.

   The culture surface will be fully dry after 2 hours.

   Plates can be used immediately once dry or can be stored dry at 4°C. For storage at 4°C, tightly wrap the vessel with Parafilm™ film and use within one week of coating.

Culture neurons

1. Isolate primary rat neurons or thaw cryopreserved primary rat neurons according to standard laboratory procedure or instructions supplied with the cells; see "Recover cryopreserved cells".
2. Plate cells in pre-warmed (37°C) complete Neurobasal™-A/B-27™ medium (postnatal) or Neurobasal™/B-27™ medium (prenatal), at a suggested density of 160 cells/mm², or another optimized density if required.
3. Incubate the culture dish at 36°C to 38°C in a humidified atmosphere of 5% CO_2 (in air is acceptable but 9% oxygen with 5% CO_2 is preferable).
4. After 4–24 hour incubation, aspirate half of the medium and replace with same volume of fresh medium.

   Return the plate to the incubator.
5. Refeed cells every 3–4 days by removing half of the medium and replacing it with an equal volume.
6. 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™ medium without glutamate, to reduce glutamate toxicity in the culture. For postnatal neurons use Neurobasal™-A/B-27™ medium, without glutamate, supplemented with 10 ng/mL bFGF.

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

Recover cryopreserved cells

Primary neuronal cells are extremely fragile upon recovery from cryopreservation.

IMPORTANT! 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 "Coat culture plates with Poly-D-Lysine").
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).

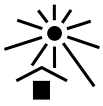
   Mix by gentle swirling after each drop.
6. Determine viable cell density using a Countess™ II Automated Cell Counter.
7. Plate ~1 × 10^5 cells per well in Poly-D-Lysine coated 48-well plate or an 8-chambered slide. Bring the cell suspension volume to 500 µL per well by adding complete Neurobasal™/B-27™ 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. See "Culture neurons" step 4–step 6.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
B-27™ Supplement (50X)	17504
B-27™ Supplement (50X) minus antioxidants	10889
B-27™ Supplement (50X) minus vitamin A	12587
G-5 Supplement (100X)	17503
N-2 Supplement (100X)	17502
GlutaMAX™ Supplement	35050
L-Glutamine, 200 mM (100X)	25030
Poly-D-Lysine	A3890401
Primary Rat Cortex Neurons	A10840
Primary Rat Hippocampus Neurons	A10841
Penicillin-Streptomycin	15070
bFGF Recombinant Human Protein	13256
2-Mercaptoethanol (1000X)	21985
DPBS, no calcium, no magnesium	14190
Countess™ II Automated Cell Counter	AMQAX1000

Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		Keep away from light
	Sterilized using aseptic processing techniques		Consult instructions for use		Caution, consult accompanying documents

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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