gibco
by life technologies

Hibernate®-A & Hibernate®-E

Description

Hibernate®-A & Hibernate®-E are serum-free, basal nutrient media for the short-term maintenance of cultured rat neurons and long-term storage of viable brain tissue in ambient CO_2 conditions. Hibernate® media give scientists greater convenience and control running benchtop live cell experiments, such as microscopy studies, flow cytometry, and other physiology studies. Hibernate®-A is designed for maintenance of postnatal and adult neural tissue and Hibernate®-E is designed for maintenance of embryonic neural tissue. Viable brain tissue can be stored for up to 1 month at 4°C in Hibernate® supplemented with B-27® Supplement before processing. Hibernate®-A or Hibernate®-E supplemented with B-27® Supplement allows for manipulations in ambient CO_2 (0.2%) after growth of neurons in Neurobasal® Medium supplemented with B-27® Supplement in a 5% CO_2 incubator. Neuron viability can be maintained for at least 2 days in Hibernate® media at ambient CO_2 levels.

Product	Catalog no.	Amount	Storage	Shelf Life*
Hibernate®-A	A12475-01	500 mL	2° to 8°C; Protect from light	24 months
Hibernate®-E	A12476-01	500 mL	2° to 8°C; Protect from light	24 months

* Shelf Life duration is determined from Date of Manufacture.

Product use

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

Important information

- Hibernate® media supplemented with B-27® Supplement can preserve viable brain tissue for up to 30 days at 2°C to 8°C.
- Hibernate® media is a suitable transport media to ship various tissues and biological specimens including umbilical cord tissue.
- Hibernate® media supplemented with B-27® Supplement is used for short-term maintenance of cultured rat neurons at 37°C in ambient CO_2 (up to 2 days) allowing convenient bench top experimentation.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare media

Neurobasal® Medium is recommended for primary rat neuron cultures. Hibernate® and Neurobasal® Media must be supplemented with L-glutamine or GlutaMAX™-I and B-27® Supplement, prior to use.

1. Aseptically add 2.5 mL/L (0.5 mM) GlutaMAX™-I.
2. Aspeptically add 20 mL/L (2%) B-27® Supplement
   Note: For primary rat hippocampus neuron cultures, the complete Neurobasal® medium requires additional supplementation of 25 µM (3.7 µg/mL) L-Glutamate up to the fourth day in culture.

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

Use

Storage of brain tissue

1. Dissect hippocampi or cortex and place one pair per 2 mL of Hibernate®-E (prenatal) or Hibernate®-A Medium (postnatal) with B-27® Supplement in a 15-mL centrifuge tube.
2. Store tube on its side, at 2° to 8°C in the dark for up to one month.
3. Remove the tissue and treat (see step 3, **Isolate neuronal cells**).

Isolate neuronal cells

The following procedure is recommended for cultured 18-day embryonic rat hippocampal or cortical neurons. Primary neuronal cells will adhere to bare plastic and glass-ware; to maximize cell recovery and yield we recommend pre-rinsing all plastic and glassware with complete Neurobasal®/B-27® medium before use.

1. Dissect cortex or hippocampi pairs from rat embryos at Day 18 of gestation (E18).
2. Collect tissue in a sterile conical tube containing Hibernate®-E complete medium. Leave the tissue in this tube (1 pair/2mL) until all dissections are completed.
3. Allow tissue to settle to the bottom of the tubes. Carefully remove supernatant leaving only the tissue covered by a minimum amount of medium.
4. Enzymatically digest the tissue in Hibernate®-E, without Ca^{2+} (BrainBits® LLC, Cat. No. HE-Ca) medium containing 2 mg/mL filter sterilized papain at 30°C for 30 minutes with gentle shaking of the tube every 5 minutes (2 pairs/mL).
5. Restore divalent cations with 2 volumes of Hibernate®-E complete medium.
6. Allow non-dispersed tissue to settle for 2 minutes and then transfer the supernatant to a sterile 15-mL conical tube and centrifuge at 150 × *g* for 5 minutes.
7. Gently resuspend the pellet in 1 mL complete Neurobasal® medium and take an aliquot for cell counting. Determine viable cell density using a Countess® Automated Cell Counter.
8. Plate ~1 × 10^5 cells (or desired cell density) per well in a poly-D-lysine coated (see **Coat Culture Surface with poly-D-lysine**) 48-well plate. Dilute cell suspension to 500 µL per well by adding Neurobasal® complete media.
9. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air. **Note:** 5% CO_2 in air is acceptable but 9% oxygen with 5% CO_2 is preferable.

Note: Many researchers favor freshly isolated neuronal cells as they maintain their functional viability, but for convenience, Gibco® Primary Rat Neurons are a flexible, ready-to-use, and quality alternative to freshly isolated neurons. Gibco® Primary Rat Neurons are isolated from day-18 Fisher 344 rat embryos and cryopreserved in a medium containing 10% DMSO.

Coat culture surface with poly-D-lysine

1. Coat culture surface (German glass or cell culture grade plastics) with a sterile 50 µg/mL solution of poly-D-lysine in water at 0.15 mL/cm^2 and incubate for one hour or overnight.
2. Rinse culture surface 2X with sterile cell culture grade water.
3. Leave the plates uncovered in a sterile environment (e.g., laminar flow hood) until dry.

Maintain neuronal cells

The following protocol can be used for freshly isolated neurons (see step 9 in **Recovery**) or cryopreserved neurons, see **Recovery**.

Recovery

Do not vortex or centrifuge cells at any time during this procedure as cells are extremely fragile upon recovery from cryopreservation. Also it is important to rinse every pipette tip and tube/vial with complete media before use to minimize cell adhesion to the plastic.

We recommend thawing one vial of cells at a time. The vial should be immediately transferred from liquid nitrogen storage to 37°C water bath minimizing handling time. A small amount of liquid nitrogen in an ice bucket can be used to transport the vials from liquid nitrogen to the water bath. Use forceps to transfer the vial.

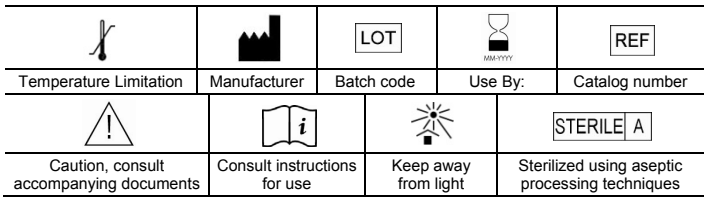
1. Rinse a 15-mL conical culture tube with prewarmed (37°C) complete Neurobasal® media and leave in the hood prior to thawing the cells.
2. If removing vial from liquid nitrogen storage, twist the cap slightly to release pressure and then retighten cap.
3. Rapidly thaw (<1 minutes) frozen vial by gently swirling in a 37°C water bath. Remove from water bath when only one tiny ice crystal is left (vial should be still cold to touch).
4. Transfer the vial to the hood and disinfect vial with 70% isopropyl alcohol. Tap vial gently on the bottom of the hood surface so that the liquid settles down to the bottom of the vial.
5. Rinse a P-1000 pipette tip with Neurobasal® complete media and very gently transfer the cells to the pre-rinsed sterile 15-mL conical tube.
6. Rinse the vial with 1 mL of prewarmed Neurobasal® complete media. Add, dropwise, one drop per second, to the cells in the 15-mL tube while gently swirling to mix. **Do not** add the full amount of media to the tube at once. This may lead to decreased cell viability due to osmotic shock.
7. Add dropwise, a further 2 mL of Neurobasal® complete media to tube (for a total suspension volume of 4 mL).
8. Determine viable cell density using a Countess® Automated Cell Counter.
9. Plate ~1 × 10^5 cells (or desired cell density) per well in a poly-D-lysine coated (see **Coat Culture Surface with poly-D-lysine**) 48-well plate. Dilute cell suspension to 500 µL per well by adding Neurobasal® complete media.
10. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air. **Note:** 5% CO_2 in air is acceptable but 9% oxygen with 5% CO_2 is preferable.
11. After 4–24 hours of incubation, aspirate half of the media from each well and replace with an equal volume of fresh prewarmed complete media. Return to incubator.
12. After 4 days in culture at 37°C in 5% CO_2 (9% O_2) remove the culture from the CO_2 incubator. Change the entire medium to prewarmed Hibernate®-E complete medium.
13. **Note:** Once placed in Hibernate® medium, cells can be maintained at 37°C for up to 2 days outside of the CO_2 incubator. Care should be taken to keep the cultures from dehydrating.

Related products

Product	Catalog no.
GlutaMAX™-I, 200 mM (100X), Liquid	35050
L-glutamine-200mM (100X), Liquid	25030
Neurobasal® Medium (1X), Liquid	21103
B-27® Serum-Free Supplement (50X), Liquid	17504
B-27® Supplement Minus AO (50X), Liquid	10889
N-2 Supplement	17502
Primary Rat Cortex Neurons, ~1 × 10^6 viable cells	A10840-01
Primary Rat Cortex Neurons, ~4 × 10^6 viable cells	A10840-02
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:



Limited Product Warranty

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For further assistance, email **techsupport@lifetech.com**