gibco
by Thermo Fisher Scientific

# B-27™ Minus Insulin

## Description

B-27™ Minus Insulin is the complete B-27™ Supplement without insulin, and it is used to support growth of hippocampal and other CNS neurons in applications where the presence of insulin causes interference.

| Product | Catalog no. | Amount | Storage | Shelf life* |
|---|---|---|---|---|
| B-27™ Minus Insulin (50X), liquid | A18956-01<br>A18956-02 | 10 mL<br>100 mL | –20°C to –5°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.

## Safety information

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

## Prepare medium

Neurobasal™-A Medium is recommended for primary neuronal cultures. Neurobasal™-A Medium requires supplementation with GlutaMAX™-I supplement (200 mM) and B-27™ Minus Insulin (50X) prior to use. Thaw B-27™ Minus Insulin overnight at 4°C.

1. Aseptically add GlutaMAX™-I to 0.5 mM final concentration (2.5 mL/L) to the medium before use.
2. Aseptically add 2% B-27™ Minus Insulin (20 mL/L) to the medium before use.

   **Note:** You may aliquot the remaining B-27™ Minus Insulin into working volumes and store at –20°C to –5°C. Thaw aliquots as needed. Do not freeze-thaw B-27™ Minus Insulin more than twice.
3. For primary rat hippocampus neuron cultures, the complete Neurobasal™-A Medium Minus Insulin (prepared from the previous steps) requires additional supplementation with 25 µM L-Glutamate up to the fourth day in culture.
4. Once supplemented, the complete Neurobasal™-A Medium Minus Insulin is stable for up to one week when stored in the dark at 2°C to 8°C.

## Use

- Use B-27™ Minus Insulin to supplement Neurobasal™ Medium for optimal viability and long-term survival of pre-natal and embryonic neuronal cells.
- Use B-27™ Minus Insulin to supplement Neurobasal™-A Medium for optimal viability and long-term survival of post-natal and adult brain neuronal cells.
- B-27™ Minus Insulin when used as a supplement to RPMI 1640 has been demonstrated to support differentiation of pluripotent stem cells into cardiomyocytes.
- Areas of application include studies of neural development, neural differentiation, diabetic neuropathy, and pluripotent stem cell differentiation.

## Cell culture procedure

The following procedure has been tested on freshly isolated 18-day gestation rat hippocampal and cortical neurons, Gibco™ Primary Rat Cortex Neurons, and Gibco™ Primary Rat Hippocampus Neurons.

1. Coat culture surface (German glass or cell culture grade plastics) with a sterile 0.05 mg/mL solution of cold poly-D-lysine in water at 0.15 mL/cm$^2$ surface area and incubate for 1 hour at ambient temperature.
2. Remove poly-D-lysine solution, and rinse twice with sterile distilled water. (Rinse thoroughly, since poly-D-lysine can be toxic to the cells). Leave the plates uncovered in the cell culture hood until the wells are completely dry. Plates can be used immediately once dry, or they can be stored dry at 4°C for up to 2 weeks.
3. Isolate primary rat neurons or thaw cryopreserved primary rat neurons according to standard laboratory procedure or instructions supplied with the cells (See **Recovery and Culture of Cryopreserved Neurons**).
4. Plate cells in pre-warmed (37°C) complete Neurobasal™-A Medium Minus Insulin (prepared as described above) at a suggested density of 160 cells/mm$^2$, or another optimized density if required.

   **Note:** For hippocampal neurons, use the complete medium supplemented with 25 µM L-Glutamate, see **Prepare Medium**.
5. 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).
6. After 4–24 hours of incubation, aspirate half of the medium and replace with same volume of fresh medium. Return the plate to the incubator.
7. **Non-hippocampal cultures:** Four days after plating, feed the cultures by aspirating half of the medium from each well and replacing with same volume of fresh medium. Repeat every three days thereafter.

   **Hippocampal cultures:** Three days after plating, replace half of the medium with complete medium **without** L-Glutamate. Repeat every three days thereafter.

   **Note:** Improved long-term survival of hippocampal neurons may be obtained by the addition of 25 µM 2-mercaptoethanol.

## Isolate primary fetal neurons

The following procedure is recommended for cultured 18-day embryonic rat hippocampal or cortical neurons.

1. Dissect cortex or hippocampi pairs from rat embryos at Day 18 of gestation (E18).
2. Collect all the tissue in a conical tube containing Hibernate-E complete medium. Leave the tissue in this tube (1 pair/2 mL) until all the dissections are completed.
3. Let the tissue settle to the bottom of the tubes and then 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 15-mL tube and centrifuge for 5 minutes at 150 × *g*.
7. Gently resuspend the pellet in 1 mL complete Neurobasal™-A Medium Minus Insulin and take an aliquot (e.g., 10 µL) for cell counting. Proceed to **Recovery and Culture of Cryopreserved Neurons**, steps 8–10.

   **Note:** Gibco™ Primary Rat Cortex and Rat Hippocampus Neurons (isolated from day-18 Fisher 344 rat embryos and cryopreserved in a medium containing 10% DMSO) are quality ready-to-use alternative to freshly isolated neurons (see **Recovery and Culture of Cryopreserved Neurons**).

## Recovery and culture of cryopreserved neurons

**Important:** 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 medium before use. **Do not** vortex or centrifuge cells at any time during this procedure as cells are extremely fragile upon recovery from cryopreservation. We recommend thawing one vial at a time. Transfer cryovial 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.

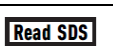
1. Rinse a sterile 15-mL conical culture tube with complete Neurobasal™-A Medium Minus Insulin and leave in the hood prior to thawing cells.
2. If removing vial from liquid nitrogen storage, twist cap slightly to release pressure and then retighten cap.
3. Rapidly thaw (<2 minutes) frozen vial by gently swirling in a 37°C water bath. Remove from water bath when only one tiny ice crystal is left (vial should still be cold to the touch).
4. Transfer the vial into the hood and disinfect with 70% isopropyl alcohol. Collect the liquid to the bottom of the vial by gently tapping the vial on the hood's surface.
5. Use a pre-rinsed P-1000 pipette tip to very gently transfer the cells to the pre-rinsed 15-mL conical tube.
6. Rinse the cryovial with 1 mL of pre-warmed complete Neurobasal™-A Medium Minus Insulin and extremely slowly add to the cells in the 15-mL tube at the rate of one drop per second. Mix by gentle swirling after each drop. 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. Slowly (dropwise) add an additional 2 mL of pre-warmed complete Neurobasal™-A Medium Minus Insulin to the tube (for a total suspension volume of 4 mL). Mix the suspension very gently with P-1000 pipette without creating any air bubbles.
8. Add 10 µL of cell suspension to a microcentrifuge tube containing 10 µL of 0.4% Trypan blue, using a pre-rinsed tip. Mix only by gently tapping the tube. Determine the viable cell density using a manual (i.e., hemocytometer) counting method. The viability of thawed cells should be >50%.
9. Plate ~1 × $10^5$ cells (see **Cell Culture Procedure**, steps 4–5) (or desired cell density) per well in a poly-D-lysine coated 48-well plate (see **Cell Culture Procedure**, steps 1–2). Dilute cell suspension to 500 µL per well by adding pre-warmed complete Neurobasal™-A Medium Minus Insulin.
10. Follow **Cell Culture Procedure**, steps 6–7, to maintain neuronal cell cultures. Incubate 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).

## Related products

| Product | Cat. no. |
|---|---|
| B-27™ Supplement (50X), liquid | 17504 |
| Neurobasal™ Medium (1X), liquid | 21103 |
| Neurobasal™-A Medium (1X), liquid | 10888 |
| RPMI 1640 Medium (1X), liquid | 11875 |
| GlutaMAX™-I (100X), liquid | 35050 |
| Hibernate E | A12476 |
| Primary Rat Cortex Neurons,<br>1 × $10^6$ viable cells/vial<br>4 × $10^6$ viable cells/vial | <br>A10840-01<br>A10840-02 |
| Primary Rat Hippocampus Neurons,<br>1 × $10^6$ viable cells/vial | <br>A10841 |
| 2-mercaptoethanol (1000X), liquid | 21985 |
| Countess™ II Automated Cell Counter | AMQAX1000 |
| Trypan Blue Stain | 15250 |

## Explanation of symbols and warnings

The symbols present on the product label are explained below:

| | | | | |
|---|---|---|---|---|
|  |  |  |  |  |
| Caution, consult accompanying documents | Temperature Limitation | Keep away from light | Sterilized using aseptic processing techniques | Consult instructions for use |
|  |  |  |  |  |
| Batch Code | Catalog number | Manufacturer | Use By: | Read Safety Data Sheet |

## 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

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

## References


1. Singh *et al*. (2012) Resistance to trophic neurite outgrowth of sensory neurons to insulin. J Neurochem *121*, 263–276.
2. Sekine *et al*. (2012) Highly efficient generation of definitive endoderm lineage from human induced pluripotent stem cells. Transpl Proceed *44*, 1127–1129.
3. Lian *et al*. (2012) Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. PNAS *109*, E1848–1857.
4. Lian *et al*. (2013) Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/β-catenin signaling under fully defined conditions. Nature Protocols *8*, 162–175.


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



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