gibco
by life technologies

PSC Neural Induction Medium

Description

PSC Neural Induction Medium is a serum-free medium that provides high efficiency neural induction of human pluripotent stems cells (PSCs) in only 7 days. Unlike existing methodologies, use of PSC Neural Induction Medium does not require the intermediary step of embryoid body (EB) formation, which adds time, labor, and variability.

Product*	Catalog No.	Amount	Storage	Shelf Life†
PSC Neural Induction Medium contains:	**A1647801**	**1 Kit**		
Neurobasal® Medium				
Neural Induction Supplement (50X)**	21103-049	500 mL	Store at 2°C to 8°C. Protect from light.	12 months
	A16477-01	10 mL	Store at –5°C to –20°C. Protect from light.	12 months

* PSC Neural Induction Medium is sold as a complete kit; its components are not available separately.

** Store Neural Induction Supplement supplement in a **non-frost-free** freezer at –5°C to –20°C.

† Shelf Life duration is determined from Date of Manufacture.

Product Use

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

Important Information

Thaw frozen Neural Induction Supplement at 2°C to 8°C overnight, or quickly in a 37°C water bath for about 5 minutes. Thawed supplement can be divided into usage-size aliquots and stored at –5°C to –20°C for the preparation of smaller volumes of complete medium. Frozen aliquots should be thawed once for use. Do not refreeze thawed aliquots.

Safety Information

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

Culture Conditions

Media: Complete PSC Neural Induction Medium

Culture Type: Adherent

Recommended Substrate: Vitronectin (Cat. no. A14700) or Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. no. A14133)

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Prepare Complete PSC Neural Induction Medium (500 mL)

1. Mix the thawed supplement by gently inverting the vial a couple of times.
2. Remove 10 mL from the bottle of Neurobasal® Medium, and then aseptically transfer the entire contents of the Neural Induction Supplement to the bottle of Neurobasal® Medium. Swirl the bottle to mix and to obtain 500 mL of homogenous complete medium.
3. Complete PSC Neural Induction Medium can be stored at 2°C to 8°C for up to 2 weeks. Before use, pre-warm the required volume of complete medium for that day in a 37°C water bath for 5–10 minutes.

Prepare Human PSC Culture for Neural Induction

1. Start with high quality human PSCs (with minimal or no differentiated colonies) cultured in feeder-free conditions, such as in Essential 8™ Medium (Cat. no. A1517001) on Vitronectin or on Geltrex® substrate. For PSC culturing protocols, visit **https://www.lifetechnologies.com/us/en/home/references/protocols/cell-culture/stem-cell-protocols/ipsc-protocols.html**.

   Note: Human PSCs cultured on mouse embryonic fibroblasts can also be used for neural induction.
2. Coat 6-well plates with the appropriate substrate on which to culture your PSCs (e.g., Vitronectin, Geltrex® matrix).
3. When PSCs reach ~70–80% confluency, remove any differentiated and partially differentiated colonies

   Note: Differentiated colonies can be marked using a Nikon® microscopy object marker (Nikon Instruments Inc., Cat. no. MBW10020) with a Nikon® microscopy C-OA 15 mm objective adapter (Nikon Instruments Inc., Cat. no. MXA20750).
4. Dislodge PSCs from culture plates following the PSC culture protocol, and estimate the cell concentration in the PSC clumps in suspension as follows:
   a. Transfer a portion of the cell suspension to a 15-mL conical tube (e.g., 1 mL of 6 mL PSC suspension prepared from one well of a 6-well plate) to estimate the total cell number of the PSC cell suspension.
   b. Centrifuge the 15-mL conical tube with the cells at 200 × *g* for 3 minutes and aspirate supernatant.
   c. Add 1 mL of pre-warmed StemPro® Accutase® cell dissociation reagent into the 15-mL conical tube with the cells and incubate for 5 minutes at 37°C.
   d. Vigorously pipette the cells up and down with a 1-mL pipette 5 times to dissociate the cells into a single cell suspension.
   e. Determine the total cell number using your preferred method. If the total cell number in 1 mL of Accutase® reagent is 1×10^6, the total number cells in the remaining 5 mL of PSC suspension is: $(1 \times 10^6) \times 5 = 5 \times 10^6$.
5. Aspirate the coating solution from the new, coated 6-well plates (from step 2), and add 2.5 mL of PSC culture medium into each well.

6. Gently shake the conical tube containing the PSC cell suspension and plate the PSCs into each well of the coated 6-well plate at 2.5×10^5–3×10^5 PSCs per well. For example, add 0.25–0.3 mL of PSC suspension into each well if the cell concentration in PSC suspension is 1×10^6 cells/mL.
7. Move the plates in several quick back-and-forth and side-to-side motions to disperse the cells across the surface and place them gently in a 37°C incubator with a humidified atmosphere of 5% CO_2.

   Note: When passaging PSCs, cells should be plated as small clumps and not as a single cell suspension. Avoid plating PSCs as single cells as that can lead to increased cell death.

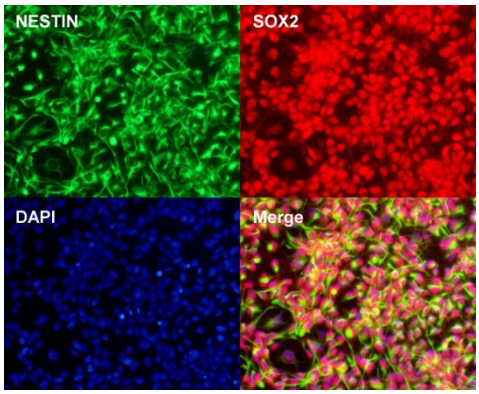
   Note: You may treat the cells overnight with 10 µM ROCK inhibitor Y27632 to prevent cell death by adding it into the PSC culture medium at the time of splitting.

Neural Induction

1. Pre-warm complete PSC Neural Induction Medium to room temperature.
2. **On day 0** of neural induction (about 24 hours after PSC splitting), PSCs should be at 15–25% confluency. Aspirate the spent medium and add 2.5 mL of pre-warmed complete PSC Neural Induction Medium into each well of 6-well plate. Return the plates into the 37°C incubator with a humidified atmosphere of 5% CO_2.
3. **On day 2** of neural induction, the morphology of cell colonies should be uniform. Mark all non-neural differentiated colonies, if any, and remove such unwanted colonies with a Pasteur glass pipette or pipette tip. Aspirate the spent medium and add 2.5 mL pre-warmed complete PSC Neural Induction Medium into each well of the 6-well plate. Return the plates into the incubator.
4. **On day 4** of neural induction, cells will be reaching confluency. Any non-neural differentiated colonies should be marked and removed. Aspirate the spent medium from each well and replace it with 5 mL of pre-warmed complete PSC Neural Induction Medium per well. Return the plates into the incubator.
5. **On day 6** of neural induction, cells should be at near maximal confluence. Remove any non-neural differentiated colonies and add 5 mL of pre-warmed complete PSC Neural Induction Medium into each well. Return the plates into the incubator.

   Note: If the color of cells turns brownish with many floating cells during day 4 to 7 of neural induction, it indicates that the starting density of PSCs was too high. In this case, change the medium every day with 5 mL of PSC Neural Induction Medium per well.
6. **On day 7** of neural induction, NSCs (P0) are ready to be harvested and expanded. For detailed instructions on NSC expansion, NSC cryopreservation, recovery of cryopreserved NSCs, and NSC characterization, refer to *Protocol: Induction of NSCs Using Gibco Neural Induction Medium*, available on our website at **www.lifetechnologies.com**.

Figure 1 Neural stem cells derived from an iPSC line using PSC Neural Induction Medium were stained for NSC markers Nestin and SOX2 using the Neural Stem Cell Immunocytochemistry Kit (Cat. no. A24354).



Related Products

Product	Cat. No.
Essential 8™ Medium	A1517001
Vitronectin, truncated human recombinant (VTN-N)	A14700
Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix	A14133
StemPro® Accutase® Cell Dissociation Reagent	A11105
Dulbecco's PBS (DPBS) without Calcium and Magnesium	14190
Human Neural Stem Cell Immunocytochemistry Kit	A24354

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

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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**

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