

PSC Neural Induction Medium

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	Package contents	Catalog number A1647801 Size 1 Kit <ul style="list-style-type: none"> Neurobasal® Medium 500 mL Neural Induction Supplement (50X) 10 mL
	Product description	<ul style="list-style-type: none"> PSC Neural Induction Medium is a serum-free medium that provides highly efficient neural induction of human pluripotent stems cells (PSCs) in only 7 days. The use of this medium does not require the intermediary step of embryoid body (EB) formation.
	Storage conditions	<ul style="list-style-type: none"> Shelf life for the kit is 12 months with proper storage. Neurobasal® Medium: Store at 2°C to 8°C. Protect from light. Neural Induction Supplement: Thaw frozen supplement at 2°C to 8°C overnight, or quickly in a 37°C water bath for 5 minutes. Store at -5°C to -20°C in a non-frost-free freezer. To prepare smaller volumes of the complete medium, divide the supplement into usage-size aliquots, and store at -5°C to -20°C. Protect from light. Do not refreeze after thawing.
	Required materials	<ul style="list-style-type: none"> 6-well plates, 15-mL conical tube, and incubator StemPro® Accutase® (Cat. no. A11105) Vitronectin (Cat. no. A14700) or Geltrex® (Cat. no. A14133)
	Timing	Supplement thawing: 5 minutes to overnight hPSC recovery: 1 day Induction: 7 days
	Selection guide	Selection Guide Related Products
	Important guidelines	<ul style="list-style-type: none"> Use 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. Cells should be plated as small clumps, not as single cells. 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 daily volume of complete medium in a 37°C water bath for 5 to 10 minutes. Click here for additional culturing guidelines for PSCs.
	Online resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support .



Protocol outline

- Prepare the complete PSC Neural Induction Medium.
- Prepare the human PSC culture.
- Perform neural induction and harvest cells.

See page 2 for instructions on preparing and running your PCR experiment.

Culture conditions

Culture type: Adherent

Recommended substrate: Vitronectin or Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix. For PSC culturing protocols, visit lifetechnologies.com.

Temperature range: 36°C–38°C

Incubator atmosphere: Humidified atmosphere of 5% CO₂. Ensure that proper gas exchange is achieved in culture vessels.

Stem cells derived using Neural Induction Medium

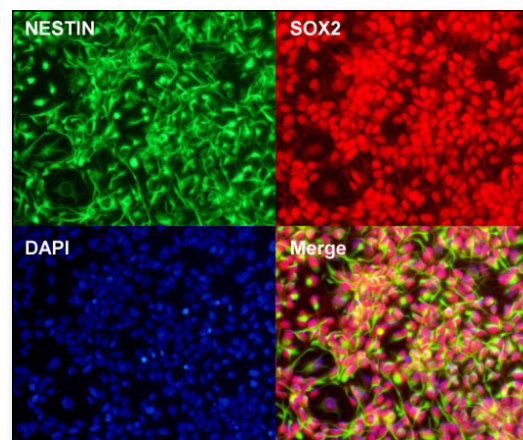



Figure: Neural stem cells derived from an iPSC line using PSC Neural Induction Medium were stained for NSC markers Nestin and SOX2 using the Neural Stem Cell Immunocytochemistry Kit (Cat. no. A24354).

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Use the following protocol to prepare the medium, initiate a culture from a frozen stock of cells, perform induction, and then harvest the cells.

Timeline		Steps	Procedure details
Day -1	1	Prepare the complete medium	<ul style="list-style-type: none"> a. Thaw the frozen Neural Induction Supplement at 2°C to 8°C overnight, or quickly in a 37°C water bath for 5 minutes. b. Mix the thawed supplement by gently inverting the vial a couple times. c. Remove 10 mL from the bottle of Neurobasal® Medium, and aseptically transfer the entire contents of the supplement vial into the bottle of medium. d. Swirl the bottle to mix and obtain 500 mL of homogenous complete medium.
	2	Split and plate PSCs	<ul style="list-style-type: none"> a. Coat 6-well plates with the appropriate substrate on which to culture your PSCs. b. When PSCs are ~70%–80% confluent, remove any differentiated and partially differentiated colonies. c. Dislodge PSCs from culture plates following the PSC culture protocol, and estimate the cell concentration of PSC clumps in suspension. d.  Click here to view the cell concentration estimation procedure. e. Aspirate the coating solution from the coated 6-well plates, and add 2.5 mL of PSC culture medium into each well. f. 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 to 3×10^5 PSCs per well. f. Move the plates in several quick back-and-forth and side-to-side motions to disperse the cells across the surface, and then place them gently into the incubator.
Day 0	3	Add complete medium to the cells	<p>Note: Wait one full day before adding medium to the cells. PSCs should be 15–25% confluent on Day 1 of PSC plating.</p> <ul style="list-style-type: none"> a. Pre-warm your complete PSC Neural Medium to room temperature. b. Aspirate the spent medium, and add 2.5 mL of pre-warmed medium into each well of the 6-well plate(s). c. Return the plates into the incubator.
Days 1–6	4	Maximize confluency	<ul style="list-style-type: none"> a. The morphology of cell colonies should be uniform by Day 2. Mark any non-neural differentiated colonies, and remove unwanted colonies with a Pasteur glass pipette or pipette tip. Aspirate the spent medium, add 2.5 mL of pre-warmed complete medium into each well of the 6-well plate, and return the plates into the incubator. b. The cells will be reaching confluency by Day 4. Mark all non-neural differentiated colonies and remove them. Aspirate the spent medium, add 5 mL of pre-warmed complete medium into each well of the 6-well plate, and return the plates into the incubator. c. The cells should be near maximal confluency by Day 6. Mark all non-neural differentiated colonies and remove them. Aspirate the spent medium, add 5 mL of pre-warmed complete medium into each well of the 6-well plate, and return the plates into the incubator.
Day 7	5	Harvest NSCs	<p>On day 8, the NSCs (P0) are ready to be harvested and expanded.</p> <p>Note: For detailed instructions on NSC expansion, cryopreservation, recovery, and NSC characterization, refer to Protocol: Induction of NSCs Using Gibco Neural Induction Medium.</p>