PSC Definitive Endoderm Induction Kit (Prototype)

Package contents

Catalog number	Size
A27654SA Buy Now	1 kit

Kit contents

Product description

PSC Definitive Endoderm Induction is a complete ready-to-use media kit for efficient induction of pluripotent stem cells (PSCs) into Definitive Endoderm (DE) lineage in two days.

Storage conditions

- Store the proudcts at –20°C to –5°C, protected from light.
- Thaw media at room temperature and use immediately or store at 2–8°C for up to 2 weeks. Avoid refreezing.

Required materials

- StemPro™ Accutase™ Cell Dissociation Reagent (Cat. no. A11105)
- Dulbecco's Phosphate Buffered Solution (Cat. no. 14190)
- UltraPure™ 0.5 M EDTA, pH 8.0 (Cat. no. 15575-020)
- Essential 8™ Medium (Cat. no. A15170-01)
- Vitronectin (Cat. no. A14700)
- RevitaCell™ Supplement (Cat. no. A26445)

Timing

- PSC subculture: 1 day
- Cell induction: 2 days

Selection guide

Selection guide

Related products

Important guidelines

- When seeding PSCs for DE induction, cells should be plated as very small clumps using the Accutase™ reagent. You can also seed PSCs as singularized cells with the TrypLE™ reagent.
- The DPBS used should be Ca^{2+} and Mg^{2+} free.
- To promote cell survival, you can treat the cells overnight with ROCK inhibitors, such as the RevitaCell™ Supplement, Y27632, or Thiazovivin.
- Ensure that hPSCs are high quality, karyotypically normal, exhibit pluripotency markers, and that passage numbers are kept below 100. If there is spontaneous differentiation in hPSCs cultures, the differentiated cells persist into protocols and confound downstream differentiation.
- Work quickly to remove cells after adding Essential 8™ Medium, as it neutralizes the initial effect of the EDTA.

Online resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.
This product is a prototype and its performance characteristics have not been established.

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Protocol outline

A. Plate PSCs in Essential 8™ Medium on Vitronectin-coated plates.
B. Feed cells with Definitive Endoderm Induction Medium A.
C. Feed cells with Definitive Endoderm Induction Medium B.
D. Characterize cells or differentiate to downstream lineages.

See page 2 to view a typical PSC induction procedure.

Culture conditions

Culture type: Adherent

Recommended substrate: Recombinant Vitronectin

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.

Single cell dissociation of PSCs for definitive endoderm induction

Passage PSCs prior to induction

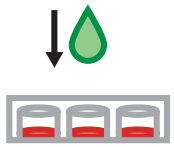
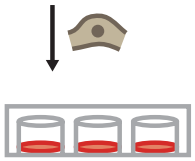
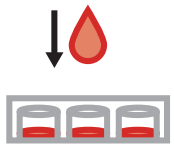
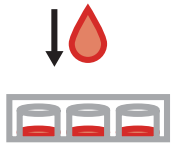
Recommended reagent volumes

Limited warranty, disclaimer, and licensing information

ThermoFisher SCIENTIFIC

Induce PSCs using the Definitive Endoderm (DE) Induction Kit

Use the following protocol to initiate PSC induction using the Definitive Endoderm Induction Kit.

Timeline		Steps	Procedure details
Prepare PSC culture	1 	**Harvest PSCs**	**Note:** On day 0 (day of splitting), the PSC culture should exhibit 70–80% confluence. **a.** Rinse the PSC culture with 1 mL DPBS (without Ca^{2+} and Mg^{2+}), aspirate, and then add 1 mL of Accutase per well of a 6-well plate. **b.** Incubate for ~5 minutes at room temperature. **c.** Collect the colonies by adding sufficient Essential 8™ Medium to the dish to dilute the original volume of Accutase™ reagent by 1:5 to 1:10. **d.** Centrifuge at 200 × *g* for five minutes at 4°C to pellet the hPSCs.
	2 	**Seed cells in Essential 8™ Medium**	**a.** Suspend the cell pellet in Essential 8™ Medium to obtain very small clumps at a split ratio (typically 1:10 from 70% confluent culture) that will achieve 15–30% confluence on the next day. **Note:** For extremely confluent hPSC cultures (> 90% of the well surface area), it is important to seed the clumps at a 1:15 to 1:30 split ratio, as the optimum range for seeding density is 0.1×10^5–0.4×10^5 cells/cm^2. **b.** Add the appropriate amount of cell suspension into the VTN-N coated 6-well plate. **Note:** To promote cell survival, at the time of splitting, you can treat the cells overnight with ROCK inhibitors, including RevitaCell™ Supplement (1X), Y27632 (10 µM), or Thiazovivin (0.5 µM). **c.** Incubate the cells overnight at 37°C in a humidified atmosphere of 5% CO_2.
Day 1	3 	**Add Induction Medium A**	**a.** Warm Induction Medium A to room temperature, shaking it several times to evenly distribute it. **b.** Ensure that the cells are **15–30%** confluent, aspirate the spent medium from the wells completely, and pipette in 2 mL of Induction Medium A per well. **c.** Incubate the solution at 37°C for 24 hours. Click here to view an example of 15–30% confluent culture. Click here to view typical Day 1 morphology.
Day 2	4 	**Add Induction Medium B**	**a.** Warm Induction Medium B to room temperature, shaking it several times to evenly distribute it. **b.** Aspirate the spent medium from the wells completely, and pipette in 2 mL of Induction Medium A per well. **Note:** It is normal to observe floating cells. A higher number of floating cells is observed if the cells are seeded at a higher density, and they should be removed before proceeding. **c.** Incubate at 37°C for 24 hours. **Note:** After the 24-hour incubation in Induction Medium B, the cells are ready to be assayed to assess their Definitive Endoderm characteristics or they can be further differentiated into downstream lineages. Click here to view typical Day 2 morphology. Click here to view an example of floating cells in the culture.