gibco
life technologies

PSC Definitive Endoderm (DE) Induction Kit (Prototype)

Description

PSC Definitive Endoderm Induction Kit is a complete ready-to-use media system for efficient induction of pluripotent stem cells (PSCs) into the Definitive Endoderm (DE) lineage in 2 days.

Product*	Catalog no.	Amount	Storage
PSC Definitive Endoderm Induction Kit (Prototype) contains:	**A27654SA**	**1 Kit**	
Definitive Endoderm Induction Medium A (Prototype)	A27652SB	50 mL	–20°C to –5°C
Definitive Endoderm Induction Medium B (Prototype)	A27653SB	50 mL	–20°C to –5°C

* The PSC Definitive Endoderm Induction Kit is sold as a complete kit; its components are not available separately.

Product use

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

Safety information

- Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
- Human origin materials are non-reactive (donor level) for anti HIV 1 & 2, anti-HCV, and HBsAg. Handle in accordance with established bio-safety practices.

Culture conditions

Culture type: Adherent

Recommended substrate: Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. no. A14700)

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.

Guidelines for differentiation

- Use high quality human PSCs that are karyotypically normal and uniformly morphologically undifferentiated. If there is spontaneous differentiation in hPSC cultures, the differentiated cells will persist into directed differentiation procedures and will confound downstream differentiation. Mechanically scrape morphologically-differentiated cells with a pipette tip daily until only undifferentiated hPSCs remain.
- Culture hPSCs under feeder-free conditions using Essential 8® Medium (Cat. no. A1517001) on VTN-N, which is an ideal substrate surface for definitive endoderm induction. For details on culturing hPSCs in Essential 8® Medium, refer to "**Culturing Pluripotent Stem Cells (PSCs) in Essential 8® Medium**".
- To promote cell survival, you can treat the cells overnight with a ROCK inhibitor such as RevitaCell™ Supplement (1X) (Cat. no. A2644501), Y27632 (10 µM), or Thiazovivin (0.5 µM) at the time of splitting.
- For routine passaging prior to induction, split the culture when the hPSC colonies occupy ~70% of the total available surface area of the well and/or when colony borders are merging with one another.
- Start definitive endoderm induction when hPSCs are at 15–30% confluence. If the culture is at a higher confluency, the cells will start detaching during induction.

Prepare DE Induction Medium

1. Thaw entire bottle of DE Induction Medium A (for use on Day 1) or DE Induction Medium B (for use on Day 2) at 4°C overnight, at room temperature (15–25°C) for ~2 hours, or at 37°C for ~20 minutes, and mix thoroughly.

   Important: Ensure that the medium is pre-warmed to room temperature before use. Cold medium will significantly disrupt cell morphology of differentiating cells.

2. Once thawed, use immediately (DE Induction Medium A on Day 1; and DE Induction Medium B on Day 2) or store at 2–8°C for up to 2 weeks.

   Alternatively, aliquot and store at –20°C. After thawing the aliquots, use immediately or store at 2–8°C for up to 2 weeks. Do not re-freeze.

Induce hPSCs into Definitive Endoderm

Day 0: Plate hPSCs

1. Aspirate the Essential 8® medium from the confluent hPSC culture and wash the cells with DPBS to remove residual media.
2. Aspirate the DPBS and add an appropriate volume of StemPro® Accutase® Cell Dissociation Reagent to fully cover the surface (at least 0.5 mL per well of a 12-well plate, 1 mL per well of a 6-well plate, or 3 mL per 10-cm dish).
3. Incubate the vessel at room temperature for ~5 minutes, continually observing the wells for cell detachment.
4. After several minutes or when some colonies begin detaching (whichever happens first), gently tap the bottom of the vessel several times. Most hPSCs colonies should freely come into suspension. If all colonies do not detach, wait 1–2 minutes, and then tap the vessel again to liberate the remaining colonies.
5. Add Essential 8® medium to the vessel to wash the colonies and dilute the StemPro® Accutase® reagent. After rinsing, collect the cell clumps in a 50-mL culture tube. Rinse the wells a second time with Essential 8® medium to ensure the recovery of all colonies.
6. Add sufficient Essential 8® medium to the 50-mL culture tube to dilute the original volume of StemPro® Accutase® reagent by 1:5–1:10.
7. Centrifuge the cell suspension at 200 × *g* for 5 minutes at 4°C to pellet the hPSCs. Carefully aspirate the supernatant, leaving the cell pellet in the culture tube.
8. Gently flick the bottom of the tube to dislodge the cell pellet.

   Note: It is important to flick the tube; otherwise, the cell pellet may be difficult to subsequently resuspend into fine clumps.

9. Resuspend the cell pellet in Essential 8® medium evenly into fine clumps by gently pipetting it up and down 2–3 times.
10. Seed the fine hPSCs clumps at ~1:10 split ratio (from 70% confluent culture) into VTN-N coated plates. Ensure that recipient wells contain sufficient final volume of Essential 8® medium (refer to Table 1).

    Important: For extremely confluent hPSC cultures (i.e., >90% confluent), it will be necessary to seed clumps at a 1:15–1:30 split ratio as the optimum range for seeding density is 0.01×10^6–0.04×10^6 cells/cm^2. Otherwise, the culture will be over-confluent post-plating and the cells will detach during induction.
11. Move the plates in several quick back-and-forth and side-to-side motions to disperse the cells across the surface and place them in a 37°C incubator with a humidified atmosphere of 5% CO_2.

    Note: To promote cell survival, you can treat the cells overnight with a ROCK inhibitor such as RevitaCell™ Supplement (1X), Y27632 (10 µM), or Thiazovivin (0.5 µM) at the time of splitting.

Day 1: Begin DE induction

1. Warm the DE Induction Medium A to room temperature. Shake the bottle several times to ensure even distribution of the components in the medium.
2. Assess the hPSCs; if the cells are 15–30% confluent, proceed with induction. If the culture is at a higher confluency, the cells will start detaching.
3. Aspirate spent Essential 8® medium from the wells completely, and add pre-warmed DE Induction Medium A (refer to Table 1).

    Note: Ensure that spent medium is completely removed before adding fresh medium.
4. Incubate the cells at 37°C for 24 hours.

Day 2: Continue DE induction

1. Warm the DE Induction Medium B to room temperature. Shake the bottle several times to ensure even distribution of the components in the medium.
2. Aspirate spent DE Induction Medium A from wells completely, and add pre-warmed DE Induction Medium B (refer to Table 1).

    Note: Ensure that spent medium is completely removed before adding fresh medium.
3. Incubate the cells at 37°C for 24 hours.

Day 3: Characterize induced cells

After 24 hour incubation of cells in DE Induction Medium B, cells will be ready to be assayed to assess for Definitive Endoderm characteristics or be further differentiated to downstream lineages.

Table 1 Reagent volumes (in mL per well or per dish)

Culture vessel (surface area)	DPBS	0.5 mM EDTA in DPBS	Complete Essential 8® medium	DE Induction medium
6-well (10 cm^2)	2 mL	1 mL	2 mL	2 mL
12-well (4 cm^2)	1 mL	0.4 mL	1 mL	1 mL
24-well (2 cm^2)	0.5 mL	0.2 mL	0.5 mL	0.5 mL
35-mm (10 cm^2)	2 mL	1 mL	2 mL	2 mL
60-mm (20 cm^2)	4 mL	2 mL	4 mL	4 mL
100-mm (60 cm^2)	12 mL	6 mL	12 mL	12 mL
T-25 (25 cm^2)	4–5 mL	2–3 mL	4–5 mL	4–5 mL
T-75 (75 cm^2)	12–15 mL	5–8 mL	12–15 mL	12–15 mL

Related products

Product	Cat. no.
Essential 8® Medium	A15170
DPBS, no calcium, no magnesium	14190
StemPro® Accutase® Cell Dissociation Reagent	A11105
Vitronectin, truncated human recombinant (VTN-N)	A14700
0.5 M EDTA	15575
RevitaCell™ Supplement (100X)	A2644501

Explanation of symbols and warnings

Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
LOT	REF		STERILE A	Read SDS
Batch Code	Catalog number	Manufacturer	Sterilized using aseptic processing techniques	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

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

Limited Use Label License No. 505: Stem Cell Intellectual Property Disclaimer

Notice to Purchaser: Products associated with this label license are not provided with a license to any patents not owned by or licensed to Life Technologies Corporation and related to stem cells. Users of Life Technologies Corporation's products subject to this label license should determine for themselves whether they have all of the appropriate licenses in place. Further, no warranty is provided that the use of these products will not infringe third party intellectual property related to stem cells.

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**.