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by Thermo Fisher Scientific

PSC Definitive Endoderm (DE) Induction Kit

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 contains:	**A30626-01**	**1 Kit**	
Definitive Endoderm Induction Medium A	A30621-01	50 mL	–20°C to –5°C, Protect from Light
Definitive Endoderm Induction Medium B	A30624-01	50 mL	–20°C to –5°C, Protect from Light

* 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.

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 persist into directed differentiation procedures and 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) or Essential 8™ Flex Medium (Cat. No. A2858501) on VTN-N, which is an ideal substrate surface for definitive endoderm induction. For more information on culturing hPSCs in Essential 8™ or Essential 8™ Flex Medium, refer to the respective user guides available for download at our website.
- 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 before 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 hPSC culture is 15–30% confluent. If the culture is at a higher confluence, 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 or 37°C before use. Cold medium will significantly disrupt cell morphology of differentiating cells.
2. After thawing, use immediately (DE Induction Medium A on Day 1, 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 refreeze.

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 any remaining media.
2. Aspirate the DPBS and add an appropriate volume of StemPro™ Accutase™ Cell Dissociation Reagent to cover the surface fully (at least 0.5 mL/well of a 12-well plate, 1 mL/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 start 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 (see Table 1).

    Important: For extremely confluent hPSC cultures (i.e., >90% confluent), seed the cell 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 back-and-forth and side-to-side motions to disperse the cells across the surface, then 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: Start DE induction

1. Warm the DE Induction Medium A to room temperature. Mix by gently inverting the bottle several times to ensure even distribution of the components in the medium.
2. Evaluate the hPSCs; if the cells are 15–30% confluent, proceed with induction. If the culture is at a higher confluence, the cells will start detaching. If this happens, start over with a fresh hPSC culture.
3. Aspirate spent Essential 8™ medium from the wells completely, and add pre-warmed DE Induction Medium A (see Table 1).

   Note: Ensure that spent medium is completely removed before adding fresh medium.
4. Incubate the cells at 37°C, 5% CO_2 for 24 hours.

Day 2: Continue DE induction

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

   Note: Ensure that the spent medium is completely removed before adding fresh medium.
3. Incubate the cells at 37°C, 5% CO_2 for 24 hours.

Day 3: Characterize induced cells

After 24 hours of incubation in DE Induction Medium B, the cells will be ready to be assayed to evaluate their 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
Essential 8™ Flex Medium Kit	A2858501
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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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