STEMdiff™ Hepatocyte Kit

Serum-free differentiation kit for generation of hepatocyte-like cells from human PSCs

Catalog #100-0520 1 Kit

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Product Description

STEMdiff™ Hepatocyte Differentiation Kit is a three-stage, serum-free differentiation kit that supports the two-dimensional differentiation of human pluripotent stem cells (hPSCs) cells to hepatocyte-like cells (HLCs). Differentiated cells express several key hepatocyte markers such as albumin and alpha-1 antitrypsin. HLCs derived using this kit can be used for various applications, including modeling human liver development and disease, drug screening and toxicity, and preclinical cell therapy studies. This kit is compatible with hPSCs maintained in mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #100-0276), or TeSR™-AOF (Catalog #100-0401).

Product Information

The following components are sold as a complete kit (Catalog #100-0520) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Endoderm Basal Medium (Hepatic)	100-0521	100 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Definitive Endoderm 100X Supplement MR	05112	0.35 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Definitive Endoderm 100X Supplement CJ	05113	1.1 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Hepatic Progenitor Medium	100-0522	100 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Hepatocyte Medium	100-0523	150 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
mTeSR™1 OR mTeSR™ Plus OR TeSR™-AOF	85850 OR 100-0267 OR 100-0401
24-well tissue culture-treated plate OR 96-well tissue culture-treated plate	e.g. 38017 OR e.g. 27136
Y-27632	72302
CellAdhere™ Laminin-521	77003
D-PBS with calcium and magnesium	Thermo Fisher 14040133
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	100-0485
15 mL Conical tubes	e.g. 38009
Trypan Blue	07050

Preparation of Reagents and Materials

Use sterile technique when preparing the following materials and media. If preparing volumes other than the indicated examples, adjust accordingly.

A. Coating Plates with CellAdhere™ Laminin-521

1. One day before seeding for differentiation, thaw one vial of CellAdhere™ Laminin-521 at 2 - 8°C.

   NOTE: If not used immediately, store at 2 - 8°C for up to 3 months.
2. Dilute CellAdhere™ Laminin-521 in ice-cold Dulbecco's phosphate-buffered saline (D-PBS) with calcium and magnesium to a final concentration of 8 µg/mL. Keep solution on ice.
3. Gently mix the diluted CellAdhere™ Laminin-521. Do not vortex.
4. Immediately add diluted CellAdhere™ Laminin-521 to a tissue culture-treated plate as follows:
   - 24-well plate: 0.25 mL diluted CellAdhere™ Laminin-521 per well
   - 96-well plate: 50 µL diluted CellAdhere™ Laminin-521 per well

   Gently rock the plate back and forth to spread the CellAdhere™ Laminin-521 solution evenly across the entire surface.
5. Seal the cultureware to prevent evaporation of CellAdhere™ Laminin-521 (e.g. with Parafilm®). Incubate at 2 - 8°C overnight.

   NOTE: If not used immediately, plates can be stored at 2 - 8°C for up to 4 weeks after coating. Do not allow the culture surface to dry, as the matrix will become inactivated.

B. Medium 1 and Medium 2

Use sterile technique to prepare Medium 1 (STEMdiff™ Endoderm Basal Medium [Hepatic] + STEMdiff™ Definitive Endoderm 100X Supplement MR + STEMdiff™ Definitive Endoderm 100X Supplement CJ) and Medium 2 (STEMdiff™ Endoderm Basal Medium [Hepatic] + STEMdiff™ Definitive Endoderm 100X Supplement CJ).

1. Thaw STEMdiff™ Definitive Endoderm Basal Medium (Hepatic) overnight at 2 - 8°C.

   NOTE: If not used immediately, store at 2 - 8°C for up to 2 months. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-freeze.
2. Thaw STEMdiff™ Definitive Endoderm 100X Supplement MR and Supplement CJ on ice.

   NOTE: If not used immediately, aliquot and store supplements at -20°C. Do not exceed the shelf life of the supplements. After thawing the aliquoted supplements, use immediately. Do not re-freeze.
3. Prepare media by combining components as indicated in Table 1.

Table 1. Preparation of Medium 1 and Medium 2 for 2 x 24-Well Plates or 2.5 x 96-Well Plates

MEDIUM	COMPONENT	VOLUME	PREPARATION & STORAGE
Medium 1 (25 mL)	STEMdiff™ Endoderm Basal Medium (Hepatic)	24.5 mL	Mix thoroughly. If not used immediately, store at 2 - 8°C for up to 1 day.
	STEMdiff™ Definitive Endoderm Supplement MR	0.25 mL	
	STEMdiff™ Definitive Endoderm Supplement CJ	0.25 mL	
Medium 2 (75 mL)	STEMdiff™ Endoderm Basal Medium (Hepatic)	74.25 mL	Mix thoroughly. If not used immediately, store at 2 - 8°C for up to 3 days.
	STEMdiff™ Definitive Endoderm Supplement CJ	0.75 mL	

C. STEMdiff™ Hepatic Progenitor Medium

Thaw STEMdiff™ Hepatic Progenitor Medium at 2 - 8°C overnight. If not used immediately, store at 2 - 8°C for up to 3 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing aliquots, use immediately. Do not re-freeze.

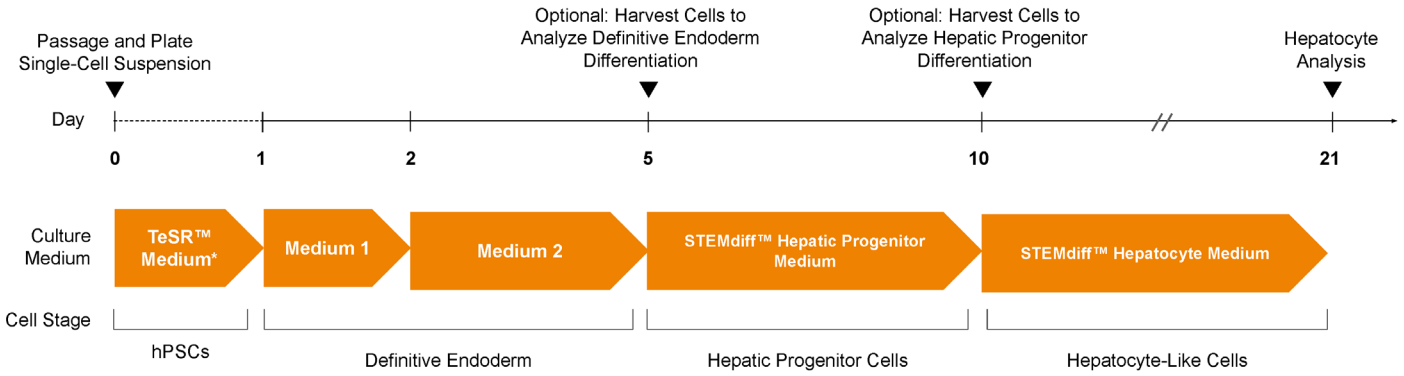
NOTE: If desired, add antibiotics (1%) to the medium.

D. STEMdiff™ Hepatocyte Medium

Thaw STEMdiff™ Hepatocyte Medium at 2 - 8°C overnight. If not used immediately, store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the medium. After thawing aliquots, use immediately. Do not re-freeze.

NOTE: If desired, add antibiotics (1%) to the medium.

Protocol Diagram



*mTeSR™1, mTeSR™ Plus, or TeSR™-AOF

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols:

A. Seeding hPSCs for Differentiation

B. hPSC Differentiation to Hepatocyte-Like Cells (HLCs)

A. Seeding hPSCs for Differentiation

The following protocol is for seeding hPSCs for differentiation to HLCs. hPSCs can be maintained in mTeSR™1, mTeSR™ Plus, or TeSR™-AOF on CellAdhere™ Laminin-521; for further information on maintaining high-quality hPSCs, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, or TeSR™-AOF, available at www.stemcell.com or contact us to request a copy. If hPSCs are maintained on Corning® Matrigel®, they should be adapted to Laminin-521 before seeding. Day 0 (seeding) can be scheduled for 6 - 8 days from the last passage of hPSCs. In the protocol below, use the medium with which the cells were maintained.

Day 0: Seeding hPSCs

The following protocol is for harvesting hPSCs from a 6-well plate and seeding into a 24- or 96-well plate. If using other cultureware, adjust accordingly.

1. One day before seeding, coat a 24- or 96-well tissue culture-treated plate with CellAdhere™ Laminin-521 (Preparation section A).
2. Prepare single-cell passaging medium by adding Y-27632 to maintenance medium (mTeSR™1, mTeSR™ Plus, or TeSR™-AOF) to a final concentration of 10 µM. Warm medium to room temperature (15 - 25°C).
3. Remove CellAdhere™ Laminin-521 solution from the plate prepared in step 1. Immediately replace the coating solution with single-cell passaging medium (prepared in step 2) as follows:
   - **24-well plate**: 0.5 mL single-cell passaging medium per well; incubate at 37°C until use
   - **96-well plate**: 0.1 mL single-cell passaging medium per well; incubate at room temperature until use

   NOTE: Ensure that the coated surface is not disturbed.
4. Use a microscope (4X magnification) to visually identify regions of differentiation in the hPSC culture and mark them using a felt tip or lens marker on the bottom of the plate.
5. Remove regions of differentiation by scraping with a pipette tip or by aspiration. Avoid having the culture plate out of the incubator for more than 15 minutes at a time.

   NOTE: Removal of differentiated cells will result in improved differentiation efficiency.
6. Wash each well with 1 mL of room temperature D-PBS (Without Ca++ and Mg++). Remove D-PBS.
7. Add 1 mL of Gentle Cell Dissociation Reagent to each well. Incubate at 37°C for 8 - 10 minutes.
8. Harvest hPSCs by gently pipetting up and down with either a 1 mL pipettor or a 2 mL serological pipette (e.g. Catalog #38002) to achieve a single-cell suspension. Transfer the single-cell suspension to a 15 mL conical tube.
9. Rinse wells with 1 - 2 mL of maintenance medium and add the rinse to the tube containing the cells.
10. Perform a viable cell count using Trypan Blue and a hemocytometer.
11. Centrifuge hPSCs at 300 *x g* for 5 minutes at room temperature.
12. Discard supernatant and resuspend hPSCs in an appropriate volume of single-cell passaging medium (prepared in step 2).

13. Seed hPSCs in the plate prepared in step 3, as follows:
    - 24-well plate: 4 x 10^5 cells per well; move the plate in several quick, short, back-and-forth and side-to-side motions to evenly distribute the cells across the surface
    - 96-well plate: Transfer the cell suspension (prepared in step 12) to a sterile reagent reservoir. Using a multi-channel pipettor, seed hPSCs at 7 x 10^4 cells per well. Allow cells to settle by leaving the plate undisturbed inside the biosafety cabinet for 1 hour.

    NOTE: Additional wells may be seeded to assess the formation of definitive endoderm on day 5, and for hepatic progenitor differentiation analysis on day 10.
14. Place the plate in a 37°C incubator. Incubate at 37°C and 5% CO_2 for 24 hours. Proceed to section B.

    NOTE: After 24 hours, observe the cultures under a microscope. The seeded monolayers should be ≥ 80% confluent before proceeding to section B. If the cultures are not confluent enough, perform a full-medium change with maintenance medium and incubate the plate at 37°C and 5% CO_2 for an additonal 24 hours before proceeding to section B.

B. hPSC Differentiation to Hepatocyte-Like Cells (HLCs)

Warm all media to room temperature (15 - 25°C) before use. The following protocol is for differentiation of hPSCs in a 24-well tissue culture-treated plate. For differentiation in a 96-well tissue culture-treated plate, perform full-medium changes using 0.1 mL of the indicated medium per well.

1. On day 1, prepare Medium 1 and Medium 2 (Preparation section B). Refer to Figure 1 for representative images of the starting confluence of hPSC cultures.

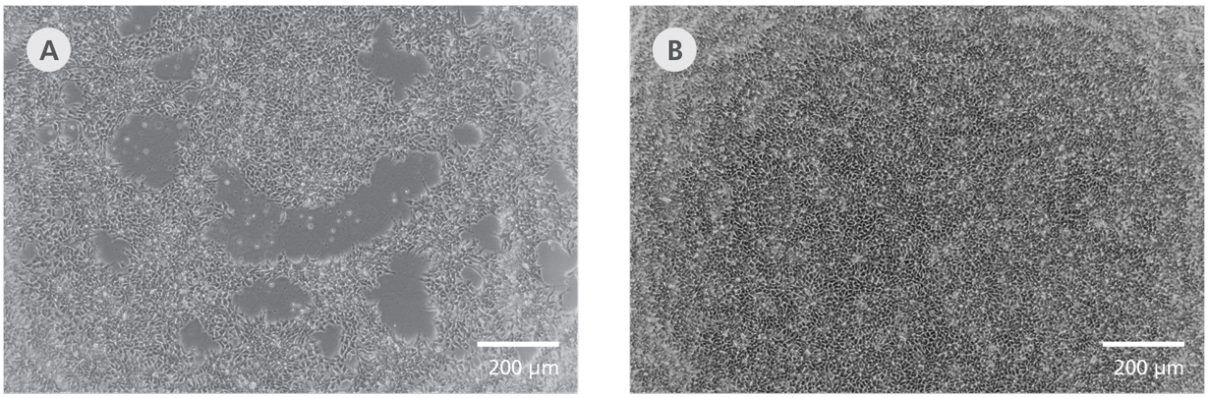


FIGURE 1. Day 1 hPSC Cultures

Day 1 hPSC cultures at **(A)** 80% confluence and **(B)** 100% confluence. Proceed with differentiation to HLCs when cultures reach 80 - 100% confluence.

2. Perform a full-medium change with 0.5 mL of Medium 1 per well of the hPSC culture. Incubate at 37°C for 24 hours.
3. On day 2, perform a full-medium change with 0.5 mL of Medium 2 per well. Incubate at 37°C for 24 hours.
4. On days 3 and 4, perform a full-medium change with 0.5 mL of Medium 2 per well. Incubate at 37°C.
5. On day 4, thaw STEMdiff™ Hepatic Progenitor Medium (Preparation section C) at 2 - 8°C overnight, for use on day 5.
6. On day 5, cells may be assayed for the formation of definitive endoderm or carried forward to hepatocytic differentiation. Refer to Figure 2A for a representative image of definitive endoderm cells on day 5.

   NOTE: Purity of definitive endoderm can be measured by flow cytometry after labeling with fluorochrome-conjugated anti-CXCR4 (e.g. Anti-Human CD184 [CXCR4] Clone 12G5, Catalog #60089) and anti-c-Kit (e.g. Anti-Human CD117 [c-Kit] Antibody, Clone 104D2, Catalog #60087) or anti-SOX17 antibodies, or by qPCR (*FOXA2*, *SOX17*, *CXCR4*, etc.).
7. Perform a full-medium change with 0.5 mL of STEMdiff™ Hepatic Progenitor Medium per well. Incubate at 37°C.
8. On days 6 and 7, perform a full-medium change with 0.5 mL of STEMdiff™ Hepatic Progenitor Medium per well. Incubate at 37°C.
9. On day 9, perform a full-medium change with 0.5 mL of STEMdiff™ Hepatic Progenitor Medium per well. Thaw STEMdiff™ Hepatocyte Medium (Preparation section D) at 2 - 8°C overnight, for use on day 10.
10. On day 10, wells may be harvested for hepatic progenitor differentiation analysis or carried forward for generation of HLCs. Refer to Figure 2B for a representative image of hepatic progenitor cells on day 10.

    NOTE: Hepatic progenitor differentiation efficiency can be assessed through morphology scoring, qPCR (*AFP*, *HNF1B*, *CK19*, *HNF6*, *TBX3*, etc.), and flow cytometry (% AFP^+, % $HNF1B^+$, and % $CK19^+$ cells).
11. Perform a full-medium change with 0.5 mL of STEMdiff™ Hepatocyte Medium per well. Incubate at 37°C for 2 days.

12. On **day 12** and every 2 days thereafter, perform a full-medium change with 0.5 mL of STEMdiff™ Hepatocyte Medium per well. Incubate at 37°C.
13. On **day 21**, cells are ready to be analyzed for hepatocyte differentiation. Refer to Figure 2C for a representative image of hepatocyte-like cells at the end of differentiation on day 21.

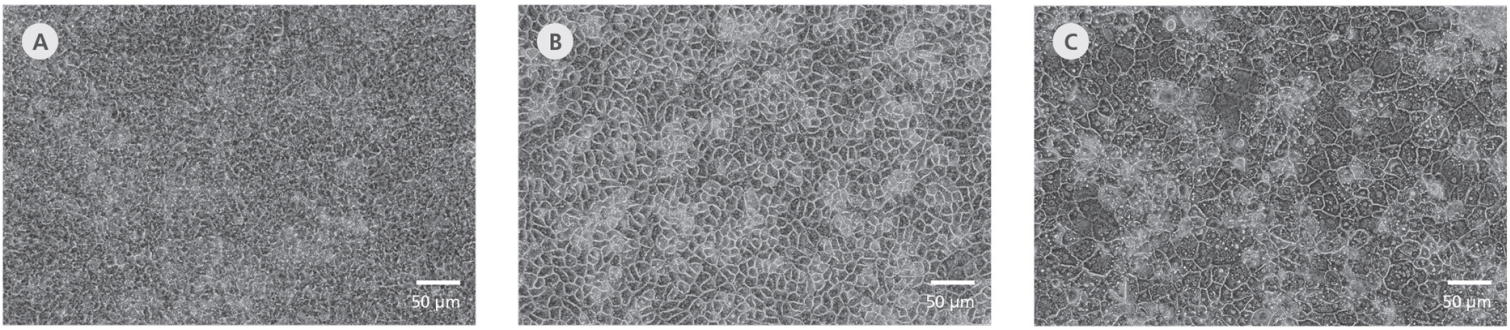


FIGURE 2. Representative Cell Morphology on Days 5, 10, and 21

(A) Day 5 definitive endoderm cells, **(B)** day 10 hepatic progenitor cells, and **(C)** day 21 hepatocyte-like cells (HLCs).

Assessment of HLCs

Hepatocyte differentiation efficiency can be assessed on day 21 through functional assays (Promega P450-Glo™ CYP3A4 Assay, ALB ELISA, etc.), qPCR (ALB, A1AT, HNF4a, CYP3a4, ASGR1, etc.), and flow cytometry (% ALB+ and % A1AT+).