CELLStart™ Substrate

Catalog Number A1014201

Pub. No. MAN0007326 Rev. 2.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

CELLStart™ Substrate is a defined substrate, containing only components of human origin (xeno-free). CELLStart™ Substrate supports human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) attachment and expansion of undifferentiated colonies in serum-free medium without the need for feeder cells. CELLStart™ Substrate can also be used with mesenchymal stem cells, neural stem cells, and for the attachment of human feeder cells (e.g., foreskin fibroblasts).

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
CELLStart™ Substrate	A1014201	2 mL	2°C to 8°C; Protect from light	12 months

[1] Shelf Life duration is determined from Date of Manufacture.

Safety information

Human origin raw materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HBsAg. Handle in accordance with established bio-safety practices.

Procedural guidelines

- Do not freeze.
- Avoid vortexing and excessive agitation since this may cause gelling. Product performance is not affected if gelling is observed.
- Collagenase is not recommended for dissociation of cells grown on CELLStart™ Substrate-coated culture containers.

Use the CELLStart™ Substrate

The following coating procedure is optimized for hESC and hiPSCs.

1. Dilute CELLStart™ Substrate 1:50 in Dulbecco's Phosphate Buffered Saline (DPBS CTS™) with calcium and magnesium.

   Note: Some cell lines may require greater dilution of CELLStart™ Substrate, we recommend optimal dilution be empirically determined for each cell line of interest.

2. Add diluted CELLStart™ Substrate to culture plates at a final volume per surface area of 78 µL/cm^2. Refer to table for respective culture container:

Culture Vessel	Surface Area (cm^2)	Volume of Diluted CELLStart™ Substrate
60-mm plate	28.25	2.25 mL per plate
6-well plate	9.6	750 µL per well
12-well plate	3.2	250 µL per well
24-well plate	2.0	160 µL per well

3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for 1–2 hours. We recommend coating culture vessels the day of use or the day before. If precoating the day before use, store the coated culture vessels at 4°C sealed with Parafilm™ to avoid drying.

4. Aspirate diluted CELLStart™ Substrate solution from culture container and discard. Culture vessel is ready for the addition of cells.

   Note: It is not necessary to rinse the culture container after removal of CELLStart™ Substrate. The bottom of the coated culture container should have a clear and wet appearance.

5. Cells can be passaged directly into KnockOut™ SR XenoFree medium (supplemented with KnockOut™ SR Growth Factor Cocktail CTS™) on CELLStart™ Substrate-coated culture containers.

   or

   Cells can be passaged directly into complete StemPro™ hESC SFM on CELLStart™ Substrate-coated culture containers.

Passage hESC cells on CELLStart™ Substrate coated plates

We recommend using either of two methods to passage cells grown on CELLStart™ Substrate coated surfaces:

1. StemPro™ EZPassage™ method or
2. TrypLE™ Select passaging method.

CELLStart™ Substrate is also compatible with other manual passaging techniques used as standard protocol.

Note: Lower plating efficiencies may be observed initially with some hESC lines when transitioning cultures to CELLStart™ Substrate.

Passage cells using the StemPro™ EZPassage™ method

Use this procedure to cut stem cell colonies using the StemPro™ EZPassage™ Disposable Cell Passaging Tool.

1. *(Optional)* Dissect out the differentiated portions of human embryonic stem cell culture using 21½ gauge needle and remove them by changing the medium (step 2 on page 2).

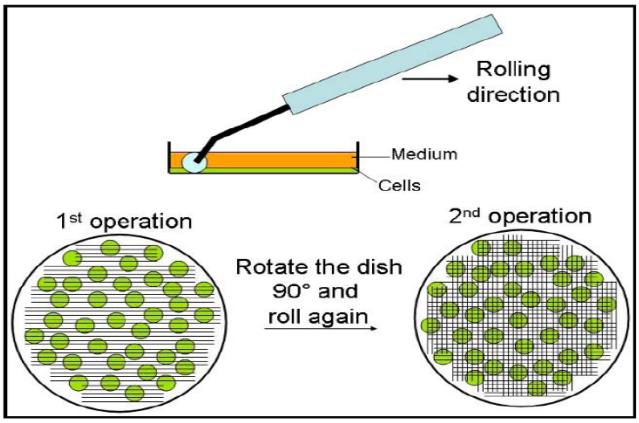
2. Aspirate the medium and wash once with DPBS CTS™. Gently add 2 mL pre-warmed complete medium to each well of 6-well plate, 3 mL per 60-mm dish.

3. Under aseptic conditions in a laminar flow hood remove StemPro™ EZPassage™ Disposable Cell Passaging Tool from packaging.

4. Hold the culture vessel in one hand and pull (roll) the passaging tool across the plate in one direction (left to right). Apply enough pressure so the entire roller blade touches the plate and maintain uniform pressure while rolling. Do not remove culture medium before rolling the plate.

5. Repeat pulling (rolling) the passaging tool parallel to the first pass until you have covered the entire plate, before moving to the next step. (see figure)

6. Rotate the culture vessel 90°, repeat steps 4 on page 2 and 5 on page 2. Discard passaging tool after use. Do not re-use. (see figure)



7. Use a cell scraper to gently detach the cut colonies from the plate. Transfer medium containing colonies to a sterile 50-mL tube. Rinse the plate with pre-warmed complete medium (1 mL/well of 6-well plate, 2 mL per 60-mm dish) collect residual cells and combine in the 50-mL tube.

   Note: Do not triturate or break cell clumps into small pieces, this may result in a single cell suspension and will lead to differentiated colonies when passaged.

8. Dilute colony suspension with pre-equilibrated complete growth medium and transfer to new coated culture vessels (typically at a 1:4 passaging rate).

Passage the cells using the TrypLE™ select passaging method

1. Aspirate spent media and rinse cells with a balanced salt solution without calcium and magnesium (i.e., DPBS CTS™, PBS, HBSS)

2. Add room temperature TrypLE™ Select to the culture container to cover the cell surface (e.g., 1 mL of TrypLE™ per 60-mm petri dish). Gently swirl the culture vessel to ensure complete coverage of the culture surface.

3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for 3 minutes. Remove the vessel from the incubator.

   Note: Longer incubation periods (>3 minutes) should be avoided. Depending on the hESC line, this may result in a single cell suspension and will lead to differentiated colonies when passaged.

4. After >90% of cells have detached, dilute cells with dilution medium (e.g., growth medium or basal medium such as DMEM/F12).

5. Transfer cell suspension to a sterile 15-mL conical tube, centrifuge at 200 × *g* for 2 minutes at room temperature.

6. Aspirate supernatant and discard. Flick the bottom of the tube with your finger a few times to disperse the cell pellet.

7. Re-suspend the colonies with pre-equilibrated complete growth medium. Cell colonies should be at the optimal size for passaging. Do not triturate into a single cell suspension.

8. Gently mix the cell suspension and transfer cells to new coated culture vessels at required passaging ratio.

Related products

Product	Catalog no.
DPBS CTS™ without Calcium Chloride without Magnesium Chloride	A12856
DPBS CTS™ with Calcium Chloride and Magnesium Chloride	A12858
TrypLE™ Select CTS™ (1X), liquid, without Phenol Red	A12589
2-Mercaptoethanol (1000X), liquid	21985
GlutaMAX™-I CTS™	A12860
StemPro™ hESC SFM	A10007-01
StemPro™ MSC SFM XenoFree	A10675-01
StemPro™ EZPassage™ Disposable Cell Passaging Tool	23181-010
KnockOut™ DMEM CTS™ (1X), liquid	A12861
KnockOut™ SR XenoFree CTS™	A10992
KnockOut™ SR GF Cocktail CTS™	A14486-01
KnockOut™ XenoFree ESC/iPSC CTS™ Kits	A14487-01 A14488-01
KnockOut™ DMEM/F-12 CTS™	A13708

Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		Read Safety Data Sheet
	Caution, consult accompanying documents		Consult instructions for use		
	Sterilized using aseptic processing techniques		Keep away from light		

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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.