GIBCO
invitrogen cell culture

CELLstart™ CTS™

Defined, humanized substrate for cell culture

CELLstart™ is a defined substrate, containing only components of human origin (xeno-free). CELLstart™ 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™ can also be used with mesenchymal stem cells, neural stem cells, and for the attachment of human feeder cells (e.g. foreskin fibroblasts).

Description	Cat. No.	Size
CELLstart™ CTS™ Defined, humanized substrate for cell culture	A10142-01	2 mL

Intended Use

For Research Use or Non-commercial Manufacturing of Cell-based Products for Clinical Research. CAUTION: Not intended for direct administration into humans or animals.

Precautions

Avoid freezing

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™-coated culture containers.

Storage

Store at 2 to 8°C. Protect from Light.

Shelf Life

12 months

Use:

Coating Procedures

The following coating procedure is optimized for human embryonic stem cells and human feeder cells.

1. Dilute CELLstart™1:50 in Dulbecco's Phosphate Buffered Saline with calcium and magnesium (Cat. No. A12858).
2. Add diluted CELLstart™ to culture container at a final volume per surface area of 0.078 mL/cm^2. Refer to table for respective culture container:

Culture Container	Surface Area (cm^2)	Volume of 1:50 Diluted CELLstart
6-well plate	9.6	750ul per well
12-well plate	3.2	250ul per well
24-well plate	2.0	160ul per well

3. Incubate in a 37°C, 5% CO_2 incubator for 2 hours. It is recommended to coat culture containers the day of use or the day before. If precoating the day before, the culture container must be stored at 2 to 8°C wrapped with Parafilm® to avoid drying.
4. Pipette out diluted CELLstart™ 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™. The bottom of the coated culture container should have a clear and wet appearance.

5. Cells can be passaged directly into STEMPRO® hESC SFM on CELLstart™-coated culture containers.

Passaging hESC Cells on CELLstart™ Coated Plates

There are two recommended passaging methods using CELLstart™: A) STEMPRO® EZPassage™ method and B) TrypLE™ Select passaging method. CELLStart™ is also compatible with other manual passaging techniques used as standard protocol.

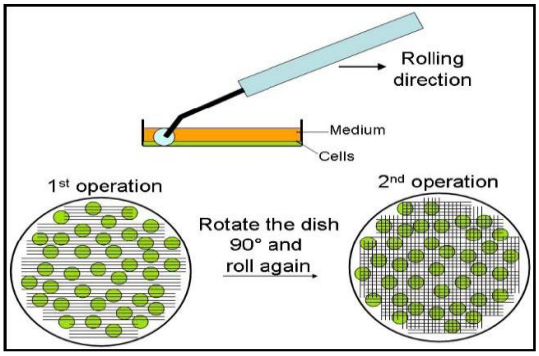
Lower plating efficiencies may be observed initially with some hESC lines when transitioning cultures to CELLstart™.

A. Manual Passaging Method Using STEMPRO® EZPassage™:

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

1. Dissect out the differentiated portions of human embryonic stem cell culture using 21½ gauge needle and remove them by changing the medium. ***Optional***
2. Pull open packaging and remove passaging tool under a laminar flow hood.
3. Hold the culture vessel in one hand and pull (roll) the passaging tool across the entire plate in one direction (left to right). Apply enough pressure so the entire roller blade touches the plate and maintain uniform pressure during the rolling action. **Do not remove the culture medium before rolling the plate.**
4. Keep pulling (rolling) the passaging tool parallel to the first pass till you have covered the entire plate, before moving to the next step. (see Figure 1)
5. Rotate the culture vessel 90°, then repeat steps 3 and 4.
6. Using a serological pipette, rinse the plate using medium on the plate so that the culture colonies are suspended in the medium.
7. Transfer the medium containing culture colonies to a 15 mL tube and spin down at 1000 rpm (200 x *g*) for 2 minutes. Alternative Method: Let tube stand to allow the colonies to settle by gravity.
8. Aspirate the supernatant carefully to remove single cells from the population.
9. Re-suspend colonies in pre-equilibrated complete growth medium and transfer to the new matrix (typically at a 1:4 passaging rate).
10. Discard passaging tool after use. Do not re-use.

Figure 1:



B. TrypLE Select Passaging Method:

1. Remove spent media from cells and rinse cells with a balanced salt solution without calcium and magnesium, i.e. PBS, DPBS, HBSS, etc.
2. Add room temperature TrypLE™ Select (Cat. No. A12859) to the culture container to cover the cell surface (e.g. 1mL of TrypLE™ per 60mm petri dish).
3. Incubate cells in a 37°C, 5% CO_2 incubator. Remove the culture container after 30 to 60 seconds and tap it against the palm of the hand a few times. Return culture container to the incubator and repeat the above process until at least 90% of cells are fully detach. This process takes approximately 3 minutes.

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 15mL centrifuge tube and spin cells down at 1000 rpm (200 x *g*) for 2 minutes at room temperature.
6. Remove supernatant and discard. Flick the bottom of the tube with your finger a few times to break up the cell pellet.
7. Re-suspend the cells with pre-equilibrated complete growth medium. Cells should be at the optimal size for passaging. Do not triturate (pipette up and down) cell suspension.
8. Immediately invert the tube a few times to get a uniform cell suspension and add cells to a new culture container at required passaging ratio.

For additional information and coating procedures for mesenchymal and neural stem cells, refer to our website:
http://www.invitrogen.com/stemcell

For additional information related to T cell expansion using Life Technologies products refer to our website:
http://www.invitrogen.com/gibcocts

Related Products

GlutaMAX™-I CTS™ (A12860)

DPBS CTS™ w/o Ca and Mg (A12856)

DPBS CTS™ w/Ca and Mg (A12858)

TrypLE™ Select CTS™ (A12859)

KnockOut™ SR XenoFree CTS™ (A10992)

KnockOut™ D-MEM CTS™ (A12861)

2-Mercaptoethanol (1,000X), liquid (21985)

FGF Basic Rec Hu (Full Length), (13256)

StemPro® hESC SFM (A10007-01)

StemPro® MSC SFM (A10332-01)

StemPro® MSC SFM XenoFree (A10675-01)

StemPro® EZPassage™ Disposable Stem Cell Passaging Tool (231810-10)

StemPro® EZChek™ Human Tri-Lineage Multiplex PCR Kit, (23191-050)

Technical Support

For additional product and technical information, such as Material Safety Data Sheets (MSDS), Certificate of Analysis, etc, please visit our website at http://www.invitrogen.com/celltherapysupport/. For further assistance, please email our Technical Support team at celltherapysupport@lifetech.com.