STEM CELL PROTOCOL

**Passaging Pluripotent Stem Cells**

The following Application Note is to give technical support for passaging human embryonic stem cells (hESC) and induce pluripotent stem cells (hiPSC) using enzymatic digest.

**A.) Passaging Pluripotent Stem Cells using Collagenase IV**

Collagenase IV is commonly used to passage human pluripotent stem cells (hESC & hiPSC). It is gentler than trypsin, but requires longer incubation times. Periodic tests to confirm karyotypic stability (Cat# GSS-2001) during long-term culturing and expansion is highly recommended.

1. Plate the feeder cells roughly 24 hours prior to passaging hESC/iPSCs.
2. Aspirate the medium from the culture flask and wash with enough 1X PBS to cover the entire cell growth area.
3. Cover the entire growth area of each flask with Collagenase IV solution (approx. 1 mg/mL) and return flask to 37°C incubator.
4. Observe hESC/iPSC colonies under the microscope after 30 minutes. Some of the hESC/iPSC colonies will start to curl up on the edges. *
5. Continue incubation until a majority of the colonies are curled up or floating. This can take between 0.5–1 h or possibly longer.
6. Add complete growth medium (ES-DMEM/F12, GSM-1002, supplemented with serum replacer and basic FGF, GSR-2001). Wash the cell growth area with a pipette to dislodge colonies from the surface.
7. Collect cells and centrifuge at 270 x g for 5 min. (Alternatively, allow the colonies to settle by gravity. This decreases the transfer of fibroblasts to the new culture.)
8. Remove most of the supernatant. Pipet the cell suspension in order to break the colonies into smaller pieces. Be careful not to pipet too much. The colonies should not be passaged to single cells.
9. Resuspend the suspension in complete growth medium.
10. Plate hESC/iPSCs into new cell culture vessel.
11. We recommend 1:3 – 1:5 split ratio depending on the growth rate of the individual cell line.

*For plates or dishes, a cell scraper can be used to detach the colonies at this point. If the colonies have started to round up at the edges, remove the Collagenase and add media. Scrape the surface and transfer the suspension to a centrifuge tube. Skip to step 7.
B.) Passaging Human Pluripotent Stem Cells Using Trypsin

Trypsin is an enzyme used to dissociate human pluripotent stem cells to smaller aggregates or single cell suspension. Extra care should be taken when using trypsin as there is some research suggesting that it may facilitate chromosomal mutation. Therefore it is crucial not to over-trypsinize the hESC/iPSC colonies and allow them to become single cells unless required by a specific assay. Periodic tests to confirm the karyotypic stability (Cat# GSS-2001) of the cell line during expansion is highly recommended.

1. Prepare flasks/dishes to receive the cells by thawing fibroblasts 24 hours before passaging. GlobalStem recommends 1:3 – 1:5 split ratio depending on the growth rate of the individual cell line.

2. Aspirate the medium from each flask/dish and wash once with 1X PBS.

3. Add enough 0.05% Trypsin/EDTA** to cover the cell growth area and quickly return flask to 37°C incubator.

4. Observe hESC/iPSC colonies under the microscope. As soon as the cells have started to round up (approx. 1 min.), tap the culture vessel gently and add equal volume serum-containing growth medium to inactivate trypsin. Do not trypsinize to single cells unless required.

5. Wash the cell growth area gently with a pipette from the top down until the sticky fibroblast layer has detached.

6. Transfer the cell suspension to a 15-mL tube. Do not break up the fibroblast sheet. Allow the fibroblast sheet to settle to the bottom of the tube and remove it.

7. Pipette the cell suspension 5–6 times.

8. Centrifuge at 270 x g for 5 minutes.

9. Remove the supernatant and resuspend in appropriate culture medium (ES-DMEM/F12, GSM-1002, supplemented with serum replacer and basic FGF, GSR-2001).

10. Aliquot the hESC/iPSCs into the new cell culture vessels dropwise.

11. Return the cells to the incubator as carefully as possible in order to insure the even distribution of the cells.

**Recombinant Trypsin can be used in place of Trypsin/EDTA and does not require serum for inactivation.