Human Pluripotent Stem Cell Passaging Protocol

This protocol is optimized for BD standard 6-well flat-bottomed tissue culture dishes. However, the protocol can be adapted to tissue culture vessels of different sizes and configurations.

Cell Passaging Steps

1. Pre-warm the required volume of cell culture medium containing 10 µM Y-27632 (final concentration) to 37°C.
   The typical volume is 2 ml for every well that the cells will be transferred to + 1 ml for each well that needs to be harvested + extra 1 - 2 ml.
   Examples:
   1. When you pass the cells from 1 well to 6 wells:
   -> [(2 ml x 6 wells) + 1 ml for harvesting + extra 1 - 2 ml] = 14 - 15 ml medium
   2. When you pass the cells from 2 wells to 8 wells:
   -> [(2 ml x 8 wells) + 2 ml for harvesting + extra 1 - 2 ml] = 19 - 20 ml medium
2. Remove the spent culture medium.
3. Wash the adherent cells gently with 2 ml of D-PBS(-) (Thermo Fisher Scientific) in each well.
4. Add 600 µl 0.5x TrypLE Select (Thermo Fisher Scientific) diluted in 0.5 mM EDTA (Sigma-Aldrich)/D-PBS(-) to each well. **See below on how to prepare 0.5 mM EDTA/D-PBS(-).*
5. Incubate the plate(s) in an incubator for 10 minutes at 37°C, 5% CO_2.
6. Aspirate the supernatant (TrypLE Select + EDTA) from each well. Wash the adherent cells remaining on the plate gently with 2 ml D-PBS(-).
7. Add 1 ml of the pre-warmed medium (37°C) with the 10 µM Y-27632 to each well.
8. Harvest the cells using a cell scraper, followed by gentle pipetting to generate a single cell suspension. **Being gentle with the scraper is critical for retaining high cell viability.**
9. Stain the cells with 10 µl of Trypan blue staining solution (Thermo Fisher Scientific). Determine the number of viable cells either by counting manually with a haemocytometer or by using an automated cell counter.
10. Adjust the concentration of the cell suspension with the pre-warmed medium containing 10 µM Y-27632. The recommended cell density is 2.0 - 5.0 x 10^4 cells/well in a 6-well plate, depending on the hPSC line used (Miyazaki et al., 2012;

Miyazaki et al., 2017). The number of cells will increase 100-fold after 6 - 7 days in culture.

11. Add an adequate volume of iMatrix-511. The recommended iMatrix-511 concentration is 0.25 µg/cm^2 (4.8 µl of 500 µg/ml iMatrix-511/well).
12. Transfer 2 ml of the cell suspension with the iMatrix-511 to each well.
13. Gently rock the plate back-and-forth and side-to-side to disperse the cells across the surface.
14. Incubate the cells for 24 hrs before exchanging the plating medium for fresh medium (without Y-27632). Replace medium on day 3 and day 5, then everyday until the next passage (usually at day 6 or day 7).

Recommended Volume of Reagents

	6 well	12 well	24 well	48 well
Approximate Area (cm^2)	9.6	4.0	2.0	1.0
0.25 µg/cm^2 iMatrix-511 (µl)	5	2	1	0.5
Medium volume (ml)	2	1	0.5	0.25
TrypLE Select/EDTA/D-PBS(-) (µl)	500	250	125	65

Preparation of 0.5 mM EDTA/D-PBS(-)

1. *First, prepare the 0.5 M EDTA/D-PBS(-) stock solution by adding 18.6 g EDTA-2Na (Sigma-Aldrich) to 100 ml D-PBS(-). Mix well until EDTA-2Na is completely dissolved. Adjust the pH to 8.0. Store at room temperature, in the dark, until needed.*
2. *To prepare the 0.5 mM EDTA/D-PBS(-) working solution, use the concentrated solution in step #1 and make a 1,000-fold dilution by adding D-PBS(-). Store it at 4°C until use. This solution is good for two weeks, any remaining solution after two weeks should be safely disposed.*