Feeding and splitting cells on mTeSR1/Cultrex

Media Preparation

mTeSR1 (500 mL): Stem Cell Technologies. Cat # 85850 (for one bottle of complete medium)
Contains FGF. Avoid heating. Aliquot and freeze or use within two weeks.
400 mL mTeSR1 basal media
+100 mL mTeSR1 5X supplement (thaw at 4°C overnight or room temperature)
+5 mL Penicillin-Streptomycin (10,000 U/mL) (optional)

Feeding hPSCs on mTeSR1/Cultrex

Feed hPSCs daily with room-temperature mTeSR1. To skip one feeding day on weekends, provide extra mTeSR1 Media the day before (up to 3 mL per well of a 6-well plate or 15 mL per 10 cm dish).

Passaging hPSCs on mTeSR1/Cultrex

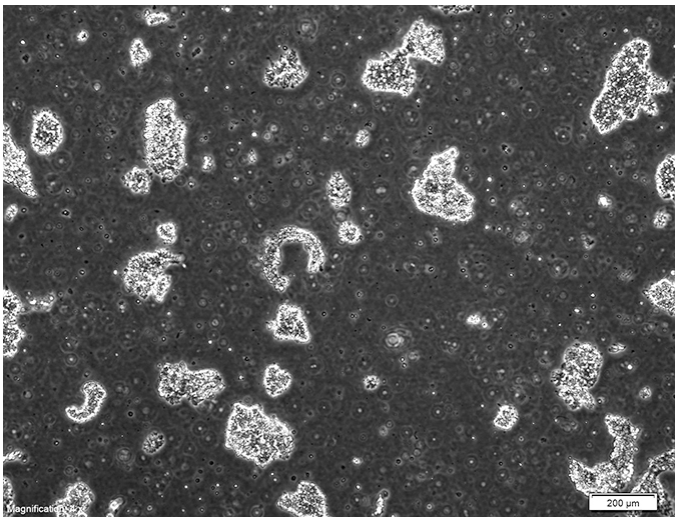
Passaging decision

The ideal time to passage feeder-free hPSCs is at 70-80% confluency. Passage at least once every 7 days to ensure the substrate stays fresh. Passage sooner if colonies show changes (e.g. cell death or differentiation in the center).
If possible, choose a passaging ratio such that cells will be at least 10% confluent 24 hours after passaging. Many feeder-free cell lines can be passaged 1:4 every 4-6 days.

Passaging Method with Gentle cell dissociation reagent (alternatively 0.5 mM EDTA or Versene can be used)

Before beginning, prepare Cultrex-coated plates or dishes.

1. Aspirate media, wash with PBS once and add Gentle Cell Dissociation Reagent (1 mL per well of a 6-well plate).
2. Incubate at room temperature for 3-5 minutes. Under the microscope, colonies should appear to separate into pieces but not detach from the plate.
3. Aspirate reagent and add mTeSR1 (1 mL per well of a 6-well plate).
4. Scrape with a cell lifter to detach colonies.
5. Pipet up and down gently to process colonies into medium-sized aggregates that are grossly uniform in size (50-200 µm; see images below). Do not create a single-cell suspension. Do not use Y-27632 (ROCK inhibitor) for routine passaging. (One pipetting up and down with 1ml pipette or 2-3 times with 5ml pipettes).
6. Add mTeSR1 to the final volume (e.g. 2 mL per well that you are splitting into).
7. Aspirate excess Cultrex from new wells and immediately plate aggregate mixture. Position plates on incubator racks and move them forward and back, then side-to-side several times to evenly distribute the cells. Do not use a circular motion.



Final size of aggregates. 4X/scale bar = 200 µm

Removing Spontaneous Differentiation

Using a picking tool and picking microscope, manually remove the spontaneous differentiation. When finished, rinse with DPBS and feed with fresh media. Check for spontaneous differentiation daily and remove it as soon as it appears.

Appendix A) Recommended materials

Product Name	**Manufacturer**	**Catalog #**
Cell Lifter	Corning	3008
Cultrex Stem Cell Qualified Reduced growth factor basement membrane extract	Trevigen	3434-005-02
mTeSR1	Stemcell Tech	85850
Gentle Cell Dissociation Reagent	Stemcell Tech	07174
Penicillin-Streptomycin (10,000 U/mL) ***(optional)***	Thermo Fisher	15140122
DPBS, no calcium, no magnesium	Thermo Fisher	14190144
alternative to mTeSR1. Stemflex	Thermo Fisher	A3349401