Transitioning to mTeSR™ Plus

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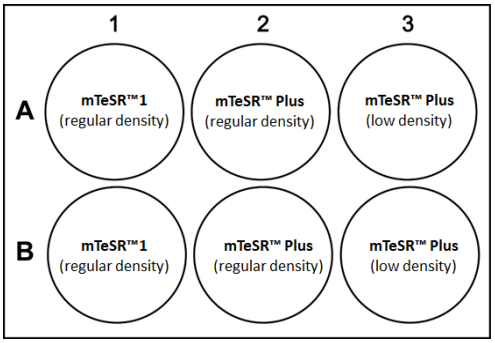
Description

The following protocol is for transitioning human ES or iPS cells from feeder-free media to mTeSR™ Plus (Catalog #05825). For complete instructions, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™ Plus, available at www.stemcell.com or contact us to request a copy. Specific sections of the manual are referenced throughout the protocol below.

Directions

1. Coat a 6-well tissue culture plate with Corning® Matrigel® (section 4.2.1) or Vitronectin XF™ (section 4.2.2). Add 2 mL medium/well to the coated plate as indicated below:



   NOTE: It is important to maintain mTeSR™1 conditions in parallel until mTeSR™ Plus cultures are established.

2. Generate a cell aggregate solution using either ReLeSR™ (section 5.1) or Gentle Cell Dissociation Reagent (section 5.2).
3. Plate the cell aggregate mixture as follows:
   - For wells in column 1: Use current mTeSR™1 plating density/split ratio
   - For wells in column 2: Use current mTeSR™1 plating density/split ratio
   - For wells in column 3: Decrease current plating density by ~25%
4. Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to side motions to distribute the cell aggregates. Do not disturb the plate for 24 hours.
5. Perform medium changes as indicated below. Visually assess cultures every 1 - 2 days to monitor growth.
   - mTeSR™1: Perform daily medium changes.
   - mTeSR™ Plus: Medium can be changed daily or every other day. To skip two consecutive days of feeding, add twice the volume of medium.
6. Based on the visual assessment (section 3.2), determine when the cultures are ready to passage. The appropriate passaging day for the mTeSR™ Plus cultures may be earlier than with mTeSR™1 cultures.
7. At the time of passaging, select the mTeSR™ Plus well that is ready to be split; maintain the split ratio of the selected well. If overgrowth is observed, decrease plating density by 20%.

   NOTE: The dissociation incubation times may need to be adjusted; dissociation with non-enzymatic passaging reagents should be monitored under the microscope until the optimal time is determined.

Once familiar with this protocol, it is possible to adjust the time at which cells are ready to be passaged by altering the cell aggregate size (section 6.3) or plating density (section 6.4).

Cells may experience an increase in spontaneous differentiation in the first 1 - 2 passages after the transition to mTeSR™ Plus. Removal of differentiated regions manually, or using specialized dissociation reagents such as ReLeSR™ (section 5.1), will help to ensure that the culture quickly adapts to the new environment without affecting the long-term health of the culture.