Splitting Human ES Cells Grown On Matrigel In TeSR1

1. Aspirate medium from plate.
2. Add 1 ml 37ºC dispase (2 mg/ml) to each well being split.
3. Incubate 5-7 minutes at 37ºC, or until edges of colonies are beginning to fold back.
4. Aspirate dispase.
5. Gently, so the colonies are not disturbed, add 2 ml pre-warmed sterile DMEM/F12 to each well, and swirl gently. Aspirate.
6. Rinse two more times to ensure all dispase is removed (any dispase remaining may dissolve matrigel, and therefore inhibit hES cell attachment).
7. Using TeSR1 medium, squirt cells off the plate (some scraping may be necessary).
8. If splitting multiple wells, combine cells to a 15 ml conical tube. As dispase is better at breaking the colonies up than collagenase, additional pipetting will likely not be necessary (if you determine that it is necessary, be gentle!).
9. Dispense colonies evenly to matrigel-coated plates.
10. Disperse cells evenly across the plate by shaking back and forth, and then side to side.
11. Place plate at 37ºC, and allow cells to attach overnight.

References:

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