Product Specification Sheet

Product Name	**EZStem™Enzyme-free Stem Cell Dissociation Solution**
Description	EZStem™ Enzyme-free Stem Cell Dissociation Solution is optimized for dissociating human embryonic or induced pluripotent stem cells that contain no enzymes and zero components of mammalian or bacterial origin. It performs exceptionally well in dissociating cell clumps while preserving the structural and functional integrity of cell surface proteins without any cytotoxic effects.
Catalog Number	M100
Size	100 ml
Shipping	Ambient Temperature
Storage and Stability	Store at 4° C upon receiving. This product is stable for 6 months when stored as directed.
Quality Control	EZStem™ Enzyme-free Stem Cell Dissociation Solution is tested for sterility and successful dissociation of human embryonic stem (hES) cells. Subsequent colony growth and morphology are monitored to ensure the cells are not affected by the treatment of EZStem™ Enzyme-free Stem Cell Dissociation Solution.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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Protocol (M100)

Using EZStem™ Enzyme-free Stem Cell Dissociation Solution for human ESC/iPSC Culture

Overview

This protocol can be used for the dissociation of human embryonic and induced pluripotent stem cells cultured with feeder cells or in feeder-free conditions. The procedure describes the dissociation of cells cultured in one well of a 6-well plate. Amounts can be scaled up if dissociating multiple wells.

Procedure

1. Culture the cells in a 6-well plate until they reach about 80% confluency.
   Note: Typically, this is around 5-6 days post-passaging.
2. Aspirate the hiPSC culture medium and rinse with DPBS (2 ml/well).
3. Add 1 ml per well of EZStem™ Enzyme-free Stem Cell Dissociation Solution and incubate at 37° C or at room temperature for 2 minutes.
   Note: Incubation times may vary between cell lines' colony sizes and the amount of dissociation solution used.
4. Carefully aspire dissociation solution, and gently rinse each well 2-3 times with 2 ml of DMEM/F-12 per well to dilute away remaining dissociation solution.
5. Add 2 ml fresh cell culture medium and lift up colonies with a cell scraper.
6. Transfer the detached cell aggregates to a 15 ml conical tube and rinse the well with an additional 2 ml fresh cell culture medium to collect any remaining aggregates. Add the rinse to the 15 ml tube.
7. Centrifuge the 15 ml tube containing the aggregates at 200 x *g* for 5 minutes at room temperature.
8. Aspirate the supernatant. Resuspend the pellet in fresh cell culture medium containing 10 µM ROCK inhibitor by gently pipetting and ensure the cell aggregates are maintained.
9. Plate the cell aggregates with 2 ml of fresh culture medium onto a new plate pre-coated with Matrigel.
   Note: If the colonies are at an optimal density, the cells can be split every 5-7 days using 1:6 to 1:10 splits.
10. Place the plate into the 37° C incubator and move the plate in quick side-to-side, forward-to-back motions to evenly distribute the clumps within the wells. Culture the cells at 37° C with 5% CO_2 and 95% humidity.
11. Change medium daily.

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