ReLeSR™
cGMP, enzyme-free human ES and iPS cell selection and passaging reagent

Catalog #  100-0484  500 mL

Product Description
ReLeSR™ is an enzyme-free reagent for dissociation and passaging of human embryonic stem (ES) or induced pluripotent stem (iPS) cells as aggregates without manual selection or scraping. Passaging human ES/iPS cells with ReLeSR™ easily generates optimally sized aggregates, while eliminating the hassle and variability associated with manual manipulation. By eliminating the need for scraping, ReLeSR™ enables the use of culture flasks and other closed vessels, thus facilitating culture scale-up and automation.

- Simple passaging protocol
- Eliminates the need for manual removal (selection) of differentiated cells
- No manual scraping to generate cell aggregates
- Compatible with passaging in flasks and large culture vessels
- cGMP, chemically defined, enzyme-free, and gentle on cells
- High expansion of human ES/iPS cells after passaging

Properties
Storage: Store at 15 - 25°C.
Shelf Life: Stable until expiry date (EXP) on label.

Directions For Use
The following protocol is for passaging human ES and iPS cells cultured in mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #05825), TeSR™2 (Catalog #05860), or TeSR™-E8™ (Catalog #05990). Volumes are listed for 6-well plates; if using alternate cultureware, adjust volumes according to surface area.

NOTE: For complete instructions on culturing ES and iPS cells, and for instructions on coating plates with Vitronectin XF™ (Catalog #07180) or Corning® Matrigel® (Corning Catalog #354277), refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #10000005505), mTeSR™ Plus (Document #10000005507), TeSR™2 (Document #10000005573), or TeSR™-E8™ (Document #10000005516). These documents are available at www.stemcell.com or contact us to request a copy.

1. At least 1 hour before passaging, coat new plates with either Vitronectin XF™ or Corning® Matrigel®.
2. Aliquot sufficient TeSR™ medium and warm to room temperature (15 - 25°C). Do not warm medium in a water bath.
3. Wash cells with 1 mL/well of D-PBS (Without Ca++ and Mg+++) (Catalog #37350) and aspirate.
   NOTE: There is no need to remove regions of differentiated cells.
4. Add 1 mL/well of ReLeSR™ and aspirate ReLeSR™ within 1 minute, so that colonies are exposed to a thin film of liquid.
5. Incubate as follows:
   - mTeSR™1 cultures: 37°C for 5 - 7 minutes
   - mTeSR™ Plus cultures: 37°C for 6 - 8 minutes
   - TeSR™2 cultures: Room temperature for 5 - 8 minutes
   - TeSR™-E8™ cultures: Room temperature for 7 - 9 minutes
   NOTE: Optimal dissociation time may vary depending on the cell line used; when passaging a cell line with ReLeSR™ for the first time, the optimal dissociation time should be determined (for more information see Figure 1 and Notes and Tips).
6. Add 1 mL/well of TeSR™ medium.
7. Detach the colonies by placing the plate on a plate vortexer (e.g. Multi-MicroPlate Genie, 120V, Scientific Industries Model SI-4000, at 1200 rpm) for 2 - 3 minutes at room temperature. Alternatively, hold the plate with one hand and use the other hand to firmly tap the side of the plate for approximately 30 - 60 seconds.
8. Transfer the detached cell aggregates to a 15 mL tube (e.g. Catalog #38009) using a 5 mL serological pipette (e.g. Catalog #38003). Cell aggregates should be appropriately sized for plating (mean aggregate size of approximately 50 - 200 µm; see Figure 1 and Notes and Tips).

   NOTE: To plate cell aggregates directly from the passaged well (i.e. without transferring into a tube), pipette the aggregate mixture up and down once using a 5 mL serological pipette. This will ensure breakup of any large aggregates that may still be present.

9. Plate the cell aggregate mixture at the desired density onto coated wells containing TeSR™ medium. If the colonies are at an optimal density, the cultures can be split every 4 - 7 days using 1 in 10 to 1 in 50 splits (i.e. cell aggregates from one well can be plated in 10 - 50 wells).

10. Place the plate in a 37°C incubator. Move the plate in several quick, short, back-and-forth and side-to-side motions to evenly distribute the cell aggregates. Do not disturb the plate for 24 hours.

   NOTE: Uneven distribution of aggregates may result in increased differentiation of human ES/iPS cells.

11. Perform daily medium changes and visually assess cultures to monitor growth until the next passaging time.

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**Figure 1.** Examples of ideal cell aggregates (mean size of approximately 50 - 200 µm) obtained after step 8 of the protocol. Images were taken using two magnifications: (A) 20X and (B) 100X. If cell aggregates do not resemble these examples, the passaging protocol may require further optimization (for more information, refer to Notes and Tips).

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**Notes and Tips**

The ideal mean cell aggregate size obtained after step 8 of the protocol is approximately 50 - 200 µm (see Figure 1). The ReLeSR™ passaging protocol may need to be optimized when using different cell lines. The following are some troubleshooting suggestions:

**LARGER AGGREGATES ARE OBTAINED** (i.e. MEAN AGGREGATE SIZE IS > 200 µm):
- Pipette the cell aggregate mixture up and down until the ideal aggregate size is obtained (see Figure 1 for example). Avoid generating a single-cell suspension.
- Increase the incubation time by 1 - 2 minutes.
- For TeSR™2 or TeSR™-E8™ cultures, increase the incubation temperature to 37°C.

**SMALLER AGGREGATES ARE OBTAINED** (i.e. MEAN AGGREGATE SIZE IS < 50 µm):
- Minimize the manipulation of cell aggregates after dissociation.
- Decrease the incubation time by 1 - 2 minutes.

**COLONIES REMAIN ATTACHED TO THE CULTUREWARE**:
- Increase the incubation time by 1 - 2 minutes.
- For TeSR™2 or TeSR™-E8™ cultures, increase the incubation temperature to 37°C.

**DIFFERENTIATED CELLS ARE ALSO DETACHING FROM THE COLONIES AFTER STEP 7**:
- Decrease the incubation time by 1 - 2 minutes.
- For mTeSR™1 or mTeSR™ Plus cultures, decrease the incubation temperature to room temperature.