

# ReLeSR™

cGMP, enzyme-free human pluripotent stem cell selection and passaging reagent

Catalog #100-0483 100 mL

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.

ReLeSR™ is manufactured following relevant cGMPs under a certified quality management system to ensure the highest quality and consistency for reproducible results. For additional quality information, refer to [www.stemcell.com/compliance](http://www.stemcell.com/compliance).

- 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

**Stability and Storage:** Store at 15 - 25°C. 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 #100-0276), TeSR™ - E8™ (Catalog #05990), or TeSR™ -AOF (Catalog #100-0401). 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 Manuals: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #10000005505), mTeSR™ Plus (Document #10000007757), TeSR™ -E8™ (Document #10000005516), or TeSR™ -AOF (Document #10000008160). These documents are available at [www.stemcell.com](http://www.stemcell.com), or contact us to request a copy.

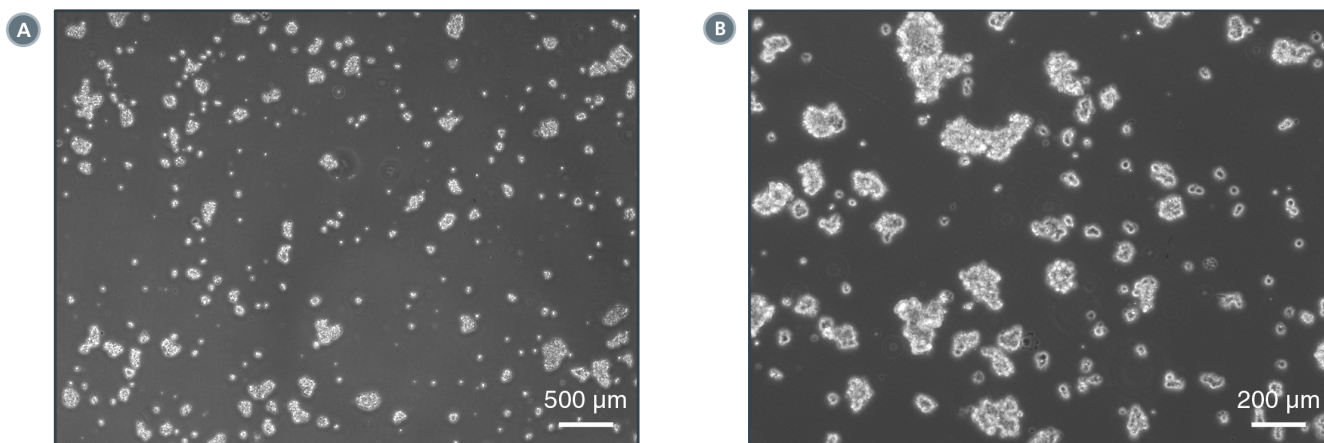
1. At least 1 hour before passaging, coat new plates with either Vitronectin XF™ or Corning® Matrigel®.
2. Aliquot sufficient TeSR™ medium and warm to room temperature (15 - 25°C). Do not warm medium in a water bath.
3. Wash cells with 1 mL/well of D-PBS (Without Ca<sup>++</sup> and Mg<sup>++</sup>; Catalog #37350) and aspirate.  
NOTE: There is no need to remove regions of differentiated cells.
4. Add 1 mL/well of ReLeSR™ and aspirate to completely remove the ReLeSR™ immediately or within 1 minute, so that colonies are exposed to only the residual liquid.

5. Incubate as follows:
  - mTeSR™1 cultures: 37°C for 5 - 7 minutes
  - mTeSR™ Plus cultures: 37°C for 6 - 8 minutes
  - TeSR™-E8™ cultures: Room temperature for 7 - 9 minutes
  - TeSR™-AOF cultures: Room temperature for 6 - 8 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  $\mu\text{m}$ ; 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.



**Figure 1. Examples of ideal cell aggregates (mean size of approximately 50 - 200  $\mu\text{m}$ ) 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).

## Notes and Tips

The ideal mean cell aggregate size obtained after step 8 of the protocol is approximately 50 - 200  $\mu\text{m}$  (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  $\mu\text{m}$ ):**

- 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™-AOF or TeSR™E8™ cultures, increase the incubation temperature to 37°C.

**SMALLER AGGREGATES ARE OBTAINED (i.e. MEAN AGGREGATE SIZE IS < 50  $\mu\text{m}$ ):**

- 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™-AOF 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.

## Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit [www.stemcell.com/hPSCworkflow](http://www.stemcell.com/hPSCworkflow) or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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