ReLeSR™

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

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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
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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 under relevant cGMPs, ensuring the highest quality and consistency for reproducible results. For additional quality information, refer to 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

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 #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.stemell.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 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 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.

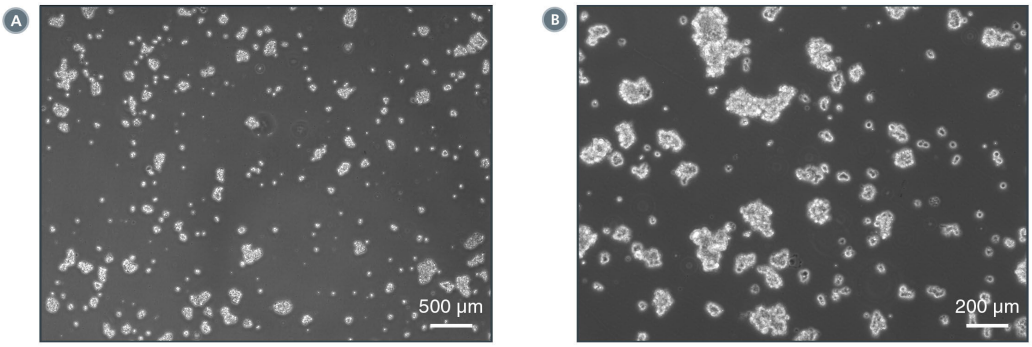

Figure 1. Examples of ideal cell aggregates (mean size of approximately 50 - 200 µm) obtained after step 8 of the protocol.
Images were taken using two magnifications: (A) 20X and (B) 100X. If cell aggregates do not resemble these examples, the passaging protocol may require further optimization. For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

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™-AOF 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™-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, or contact us at techsupport@stemcell.com.