ReLeSR™

Enzyme-free human ES and iPS cell selection and passaging reagent

Catalog # 05872 100 mL
05873 500 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

ReLeSR™ is an enzyme-free reagent suitable for the dissociation of human embryonic stem (ES) or human induced pluripotent stem (iPS) cell colonies into cell aggregates without the need for manual scraping.

- 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
- 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.

Handling / Directions For Use

The following protocol is for passaging human ES and iPS cells cultured in either mTeSR™1 (Catalog #05850), TeSR™2 (Catalog #05860), or TeSR™-E8™ (Catalog #05940). Volumes are listed for 6-well plates; if using alternate cultureware, adjust volumes according to surface area.
NOTE: Refer to the Technical Manuals: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #29106), TeSR™2 (Document #28210), or TeSR™-E8™ (Document #29267) 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 #353046). These documents are available on our website at www.stemcell.com or contact us to request a copy.

1. At least one 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
   - TeSR™2 cultures: room temperature (15 - 25°C) for 5 - 8 minutes
   - TeSR™-E8™ cultures: room temperature (15 - 25°C) 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 (15 - 25°C). 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 using a 5 mL serological pipette. 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 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 pre-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 1 well can be plated in 10 to 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 and iPS cells.
11. Perform daily medium changes and visually assess cultures to monitor growth until the next passaging time.

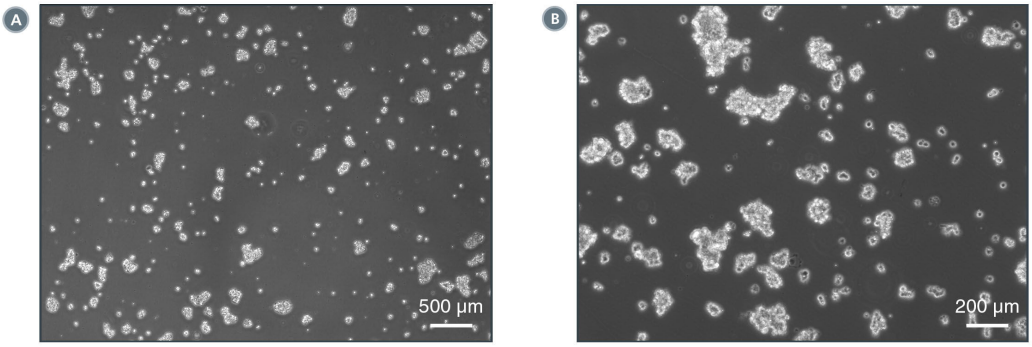

Figure 1. Examples of ideal cell aggregates (mean size of approximately 50 - 200 µm) obtained after step 8 of the protocol. Images were taken using two magnifications: (A) 20X and (B) 100X. If cell aggregates do not resemble these examples, the passaging protocol may require further optimization (for more information, refer to the section 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™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 cultures, decrease the incubation temperature to room temperature (15 - 25°C).

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.