FreSR™-S

Animal component-free medium for freezing ES and iPS cells as single cells

Catalog # 05859 50 mL

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Product Description

FreSR™-S is a defined, serum-free, and animal component-free medium for the cryopreservation of human pluripotent stem cells. This complete and ready-to-use medium is recommended for human embryonic stem (ES) or induced pluripotent stem (iPS) cells previously cultured in mTeSR™1 (Catalog #85850), TeSR™2 (Catalog #05860), or TeSR™-E8™ (Catalog #05940). Frozen cells should be stored at -135°C (liquid nitrogen) or colder.

NOTE: This product is not recommended for freezing cell aggregates.

- Defined, serum-free and animal component-free medium for freezing ES/iPS cells as single cells
- Quickly recover ES/iPS cell colonies after thawing
- Reproducibly high recovery rates
- Optimized for freezing ES/iPS cells previously cultured in TeSR™ maintenance media
- Preserves ES/iPS cell pluripotency and expansion capacities
- Convenient, ready-to-use format

Properties

Storage: Store at 2 - 8°C.
Shelf Life: Stable until expiry date (EXP) on label.

Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

Handling / Directions For Use

NOTE: For complete instructions on culturing ES and iPS cells, on preparing a single-cell suspension using Gentle Cell Dissociation Reagent (Catalog #07174), and 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 #28315), TeSR™2 (Document #28210), or TeSR™-E8™ (Document #29267). These documents are available at www.stemcell.com or contact us to request a copy.

FREEZING

1. Prepare a single-cell suspension of human ES or iPS cells using the desired dissociation reagent.
2. Perform a viable cell count using Trypan Blue (Catalog #07050) and a hemocytometer.
3. Centrifuge cells at 300 x g for 5 minutes to obtain a cell pellet.
4. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
5. Wipe the outside of the FreSR™-S container with 70% ethanol or isopropanol before opening.
6. Add cold (2 - 8°C) FreSR™-S to obtain a cell suspension of 1 x 10^6 cells/mL and mix thoroughly.
7. Transfer 1 mL of the single-cell suspension into each cryovial.
8. Freeze cells using a standard slow rate-controlled cooling protocol (approximately -1°C/minute) or an isopropanol freezing container and store at liquid nitrogen temperature (-135°C).
   NOTE: Long-term storage at -80°C is not recommended.

THAWING

1. Have all tubes warmed (15 - 25°C) desired culture medium (e.g. mTeSR™1, TeSR™2, or TeSR™-E8™) and DMEM/F-12 with 15 mM HEPES (Catalog #36254), and pre-coated cultureware ready before starting the protocol to ensure that the thawing procedure is done as quickly as possible.
2. Add Y-27632 (Catalog #72302) to desired culture medium to reach a final concentration of 10 µM.
3. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
4. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
5. Quickly thaw cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex cells.
6. Wipe the outside of the vial with 70% ethanol or isopropanol.
7. Use a 1 mL micropipette to slowly transfer the contents of the cryovial to a 15 mL conical tube (e.g. Catalog #38009) containing 5 - 7 mL of DMEM/F-12 with 15 mM HEPES.
8. Centrifuge cells at 300 x g for 5 minutes at room temperature (15 - 25°C).
9. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed.
10. Add 1 mL of desired culture medium containing 10 µM Y-27632 to the tube. Mix gently.
11. Plate cells onto pre-coated cultureware.
    NOTE: In general, one frozen cryovial containing 1 x 10^6 cells can be thawed and plated into 1 - 2 wells of a 6-well plate.
12. Place the cultureware in a 37°C incubator. Move the cultureware in several quick, short, back-and-forth and side-to-side motions to evenly distribute the cells across the surface.
13. Perform daily medium changes using desired culture medium (without Y-27632) and visually assess cultures to monitor growth until the next passaging time (i.e. 80 - 90% confluent). This takes approximately 2 - 5 days after thawing.
    NOTE: This time may vary when using different cell lines. Cultures should be monitored under the microscope until the optimal passaging time is determined.
14. Passage cultures using standard techniques to generate cell aggregates (e.g. ReLeSR™ [Catalog #05872]).
    NOTE: It is generally not recommended to perform serial single-cell passaging due to the increased risk of karyotype abnormalities (Draper JS et al.; Buzzard JJ et al.).

References

1. Buzzard JJ et al. (2004) Karyotype of human ES cells during extended culture. Nat Biotechnol 22(4): 381–2.
2. Draper JS et al. (2004) Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat Biotechnol 22(1): 53–4.