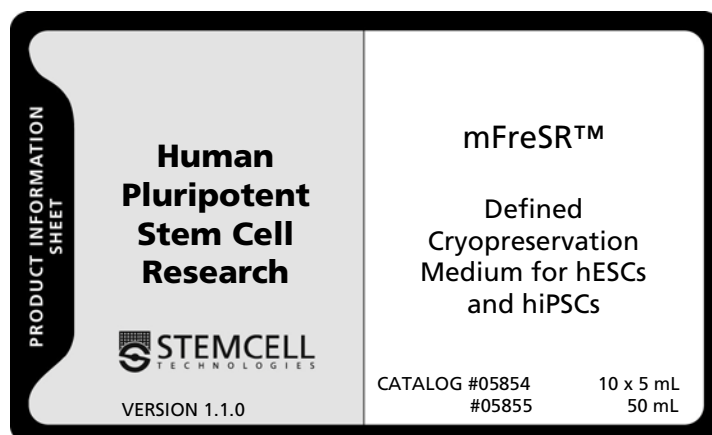


PRODUCT DESCRIPTION

mFreSR™ is for the cryopreservation of clumps of human embryonic and induced pluripotent stem cells (hESCs and hiPSCs) cultured in mTeSR™1 (Catalog #05850/05870). Cryopreserved hESCs or hiPSCs should be stored at -150°C or in the vapor phase of liquid nitrogen. mFreSR™ contains dimethylsulfoxide (DMSO) and is complete and ready-to-use.

mFreSR™ is serum-free and may be used in cryopreservation protocols requiring the use of defined medium.



STABILITY AND STORAGE

Product stable at -20°C for 1 year from date of manufacture as indicated on label.

The mFreSR™ 50 mL bottle (Catalog #05855) can be thawed, dispensed into convenient aliquots then refrozen. Avoid repeated freeze/thaw cycles.

Use thawed mFreSR™ 5 mL tubes (Catalog #05854) or aliquots on the same day to cryopreserve hESCs or hiPSCs. Do not store thawed mFreSR™ for more than 1 day.

This product has been aseptically manufactured using tightly controlled processes and is sterility tested.

RELATED PRODUCTS

PRODUCT	CATALOG #
AggreWell™400 plates for the reproducible formation of uniformly sized embryoid bodies	27845 27945
Anti-Oct 3/4 antibody	01550 01551
Anti-SSEA-1 antibody	01552
Anti-SSEA-3 antibody	01553
Anti-SSEA-4 antibody	01554
Anti-TRA-1-60 antibody	01555
Anti-TRA-1-81 antibody	01556
FITC-conjugated goat anti-mouse IgG	10210
FITC-conjugated goat anti-mouse IgM	10211
APC-conjugated goat anti-rat IgM	10215
mTeSR™1 medium for the maintenance of hESCs and hiPSCs	05850 05870
ACCUTASE®	07920
Dispase (1 mg/mL)	07923

DIRECTIONS FOR USE

Refer to the Material Safety Data Sheet for more information.

1.0 Cryopreserving hESCs and hiPSCs cultured in mTeSR™1

The following is based on hESC cultures in 6-well plates where initial clump seeding is adjusted so that plates are 60 - 70% confluent at time of cryopreservation.

Before cryopreservation, hESCs or hiPSCs should be of high quality (primarily undifferentiated). Cryopreservation should be done approximately 1 - 2 days before the usual day of passaging. hESCs and hiPSCs will have increased survival if cryopreserved as large clumps.

1. Bring required amount of mFreSR™ to room temperature (15 - 25°C).
2. In the hESC culture to be cryopreserved, use a microscope to visually identify regions of differentiation. Mark these using a felt tip marker or lens marker on the bottom of the plate.
As in routine passaging, this selection should not exceed 20% of the well if the culture is of high quality.
3. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
4. Aspirate remaining medium from wells.
5. Rinse wells with 2 mL of phosphate buffered saline (PBS; Catalog #37350), and aspirate.
6. Add 1 mL per well of dispase (Catalog #07913) at a concentration of 1 mg/mL. Place at 37°C for 7 minutes.
The time recommendation is based on STEMCELL Technologies' dispase. If using dispase from another supplier, this time may need to be adjusted. After incubation, the colony edges should appear slightly folded back but should remain attached to the plate.
7. Remove dispase and gently rinse each well 2 - 3 times with 2 mL of DMEM/F12 (Catalog #36254) per well to dilute away any remaining dispase.
8. Add 2 mL/well of DMEM/F12 or mTeSR™1 (Catalog #05850/05870) and scrape colonies off using a cell scraper or a 5 mL serological pipette.
Take care to keep the clumps as big as possible.
9. Transfer the detached cell aggregates into a 15 mL conical tube and rinse the wells with additional 2 mL DMEM/F-12 or

- mTeSR™1 to collect any remaining aggregates. Add the rinse to the 15 mL tube containing the cell aggregates.
10. Centrifuge the 15 mL tube containing the aggregates at 300 x g for 5 minutes at room temperature (15 - 25°C).
Prepare and label cryotubes for dispensing while cells are centrifuging.
 11. Remove the supernatant taking care to keep the cell pellet intact.
 12. Using a 2 mL pipette, gently resuspend the pellet in mFreSR™ medium, taking care to leave the clumps larger than would normally be done for passaging.
1 mL of mFreSR™ should be used for every well of a 6-well plate being frozen. However, if the wells are at low density (less than 50% confluent), 1 mL of mFreSR™ may be used for every 2 wells.
 13. Gently mix suspension and mFreSR™ and transfer 1 mL at a time into labelled cryovials using a 2 mL pipette.
Draw up 1 mL at a time and aliquot 1 mL/tube. This will ensure even distribution of clumps between the wells.
 14. Place vials into an isopropanol cryovessel and place the vessel at -80°C to -150°C overnight.
 15. Transfer to the liquid nitrogen vapor tank after freezing overnight.

2.0 Thawing hESCs and hiPSCs cryopreserved in mFreSR™

hESCs and hiPSCs can be thawed into either 4- or 6-well plates. If unsure of the number of clumps frozen down, the 4-well plate is recommended. Generally, hESCs or hiPSCs cryopreserved from 1 well of a 6-well plate can be thawed successfully into 1 well of a 6-well plate.

a. Preparation of BD Matrigel™ coated plates

Details can be found in the manual "Maintenance of Human Embryonic Stem Cells in mTeSR™1" (Manual Catalog #29106) available on our website at www.stemcell.com/technical/manuals.aspx.

1. Remove an aliquot of frozen BD Matrigel™ hESC-qualified Matrix (BD Catalog #354277) from -70°C. Thaw on ice and then dilute 1/25 in cold DMEM/F12.
2. Immediately use the diluted BD Matrigel™ solution to coat tissue culture-treated plates. For a 4-well plate, use 0.5 mL of diluted BD Matrigel™ solution per well. For a 6-well plate, use 1 mL of diluted BD Matrigel™ solution per well. Swirl the plate to spread the BD Matrigel™ solution evenly across the surface.
Only tissue culture-treated plates should be used for coating with BD Matrigel™.
3. Coated plates should be left at room temperature (15 - 25°C) for at least 1 hour before use. Do not allow the plates to dehydrate.
Remove BD Matrigel™ solution from plates immediately before adding medium and cells.

4. If not used immediately, the plates must be sealed (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 7 days after coating. Sealing is required to prevent dehydration.

Plates are not optimal for hESC or hiPSC culture if the BD Matrigel™ solution does not completely cover the surface; therefore, plates that have regions where the BD Matrigel™ solution has evaporated are not recommended for use.

b. Thawing cryopreserved hESCs and hiPSCs

Have all tubes, warmed medium and plates ready before starting the protocol to ensure that the thawing procedure is done as quickly as possible.

1. Remove frozen vial of cells from the liquid nitrogen vapor tank and immediately dip the tube into a beaker containing water at 37°C. Agitate the tube in the water continuously until only a small ice chunk remains.
2. Spray the tube with ethanol or isopropanol to sterilize. Transfer the contents of the tube to a 15 mL conical tube using a 2 mL pipette to minimize breaking of any clumps.
3. Dropwise, add 5 - 7 mL of warm mTeSR™1 (Catalog #05850/05870) to the tube, mixing as the medium is added.
4. Centrifuge cells at 300 x g for 5 minutes at room temperature (15 - 25°C).
5. Aspirate medium leaving the pellet intact. Using a 2 mL pipette, gently pipette the cell pellet in 1 - 2 mL of mTeSR™1 taking care to maintain the clumps as large as possible.
6. Gently tilt the plates onto one corner and allow the excess BD Matrigel™ solution to collect in that corner. Remove the BD Matrigel™ solution using a serological pipette or by aspiration. Ensure that the tip of the pipette does not scratch the coated surface.
If plates have been stored at 2 - 8°C, allow the plates to come to room temperature (15 - 25°C) for 30 minutes before removing the BD Matrigel™ solution.
7. Transfer 0.5 mL (or 2 mL) of medium plus clumps per well to a BD Matrigel™ coated 4-well (or 6-well) plate.
Ensure that clumps are evenly distributed between wells.
8. Place plate at 37°C and move the plate in quick side to side, forward to back motions to evenly distribute the clumps within the wells.
9. Change medium daily. Check for undifferentiated colonies that are ready to passage (dense centered) approximately 5 - 7 days after thawing.
If only a few undifferentiated colonies are observed after thawing, it may be necessary to select only these colonies for passaging and replate them in the same size well on a new BD Matrigel™ coated plate.

Warning - Hazardous Ingredient: DMSO. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. DMSO is readily absorbed through the skin and may carry such materials into the body. Wash exposed skin with soap and water. Flush eyes with water. For more information, refer to the MSDS.