# PluriSTEM™ Freeze Medium

## Stem Cell Medium

## Cat. # SCM134

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

**pack size: 50 mL**

Store at -20ºC

## Certificate of Analysis



### Background

PluriSTEM™ Freeze Medium is a defined, serum-free medium for the cryopreservation of human ES and iPS cells. PluriSTEM™ Freeze Medium contains dimethyl sulfoxide (DMSO) and is provided as a complete ready-to-use formulation.

### Storage and Handling

Store at -20°C until expiration date on the product label. Before use, thaw PluriSTEM™ Freeze Medium at room temperature (15 - 25°C) or overnight at 2 – 8°C. Do not thaw at 37°C. After thawing, aseptically dispense into working aliquots and store at -20ºC. Use aliquots within expiration date as indicated on label. Avoid multiple freeze thaw cycles.

### Cryopreservation Protocol for Human ES/iPS Cells

Before cryopreservation, human ES/iPS cell cultures should be of high quality (primarily undifferentiated with less than 5% of the cells being differentiated). Cryopreservation should be performed when cells are ready to be passaged. The following protocols are based on cultures in 6-well plates where wells are 70 – 80% confluent at the time of cryopreservation.

1. Thaw PluriSTEM™ Freeze Medium on ice.
2. Prepare labeled cryovials. Estimate that 1 cryovial should contain colonies from 2 wells of a 6-well plate.
3. Use a dissection microscope to visually inspect the plate containing human pluripotent cells to be passaged. Inspect the colonies for areas of spontaneous differentiation.

   ***Note:*** *Areas of spontaneous differentiation are characterized as highly phase-bright, dense areas with irregular borders, non-uniform cell morphologies and cell types and are typically localized either in the center of the colonies or along the edges between colonies.*
4. Use a sterile p200 pipette tip attached to a p200 pipetman to scrape away areas of spontaneous differentiation.
5. Aspirate the medium containing the scrapped areas from the wells. Rinse with 2 mL per well of DMEM/F12 medium or 1X PBS (Cat. No. BSS-1006-B).
6. Add 1 mL Dipsase II (1 mg/mL, Cat. No. SCM133) per well of the 6-well plate containing pluripotent human ES/iPS cells.

### Cryopreservation Protocol continued…

7. Incubate at 37°C for 6 – 7 minutes. After incubation, visually inspect the colonies under a microscope. The edges of the colonies many appear slightly rounded up and folded back but the overall colony should be attached to the plate.
8. Aspirate the Dispase II and gently rinse each well two times with 2 mL per well of DMEM/F12 medium or 1X PBS to remove any residual Dispase II solution. Aspirate after each rinse.
9. Add 1.5 – 2 mL PluriSTEM™ medium to each well. Gently detach the colonies using a cell scraper (Sarstedt Cat. No. 83.1832)
10. Use a 5 mL serological pipette to collect the cell aggregates to a 15 mL conical tube. Minimize pipetting up and down as this may break up the colonies to suboptimal small pieces. The process of transferring the cell aggregates to the 15 mL conical tube should be sufficient to break the colonies to sufficient size.
11. Rinse the wells with an additional 2 mL of PluriSTEM™ medium per well to collect any remaining cell aggregates. Add the rinse to the 15 mL conical tube.
12. Centrifuge the 15 mL conical tube containing the cell aggregates at 300 x g for 5 minutes at room temperature (15 - 25°C).
13. Aspirate the supernatant. Gently resuspend the cell pellet in the appropriate volume or cold (2 – 8°C) PluriSTEM™ Freezing Medium using a 5 mL pipette. Take care to keep the cell aggregates as big clumps.

    Note: 1 mL of PluriSTEM™ Freeze Medium may be used to freeze colonies form 2 wells of a 6-well plate. However, if the colonies are greater than 80% confluent, 1mL of PluriSTEM™ Freezing Medium may be used to freeze 1 well of a confluent 6-well plate.
14. Transfer 1 mL of the cell suspension into a labeled cryovial using a 5 mL pipette.
15. Quickly place the cryovials into an isopropanol freezing container (i.e. Mr. Frosty) and place the container at -80°C overnight.
16. Next day, transfer frozen cryovials to a liquid nitrogen vapor tank for long-term storage.

**SPECIES LEGEND:** **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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**Please visit www.millipore.com for additional product information and references.**

### Thawing Frozen Human ES/iPS cells

Human ES and iPS cells should be thawed into tissue culture-treated plates coated with 1:20 dilution of Matrigel™. Generally, one cryovial containing cells frozen in PluriSTEM™ Freezing Medium may be successfully thawed into 1 well of a matrigel-coated 6-well plate.

1. Coat new 6-well plates with 1:20 dilution of Matrigel (1.5 mL per well) . Swirl the culture plates to spreaad the Matrigel evenly across the surface of the plate. Incubate 2 - 8°C overnight or at room temperature for 1-2 hours before use.

2. On the day of thawing, acclimate matirgel coated plates for 1 hour at room temperature. After 1 hour, remove the matrigel coating. Add 2 mL PluriSTEM™ medium to each well. Set plate aside until cells are ready to be passaged.

3. Aliquot sufficient PluriSTEM™ and DMEM/F12 to culture the thawed cells. Warm reagents at room temperature (15 - 25°C) for 5 – 10 minutes.

4. Remove the vial of cryopreserved cells from liquid nitrogen storage and quickly thaw the cells in a 37°C water bath. Closely monitor until only small ice crystals remain. Quickly remove the vial from the waterbath. IMPORTANT: Do not vortex the cells or leave them in the water bath for too long.

5. Disinfect the outside of the vial with 70% ethanol or isopropanol. Proceed immediately to the next step.

6. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.

7. Using a 10 mL pipette, slowly add dropwise 9 mL of PluriSTEM™ Medium to the 15 mL conical tube. IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock.

8. Gently mix the cell suspension by slow pipeting up and down twice. Be careful not to introduce any bubbles.

### Thawing Frozen Human ES/iPS cells continued…

9. Centrifuge the tube at 300 x g for 5 minutes at room temperature (15 - 25°C).

10. Aspirate the supernatant. Resuspend the cell pellet in 1 mL of PluriSTEM™ by gently pipetting the cells up and down twice. Take care to maintain the cells as aggregates.

11. Transfer 1 mL of the thawed cell aggregates to one well of the matrigel-coated 6-well plate containing 2 mL PluriSTEM™ medium that had been set aside from step 2. Total volume per well = 3 mL.

12. Place the plate in a 37°C. Agitate the plate **gently** from side to side and forward and backwards to ensure that the cell aggregates are evenly distributed across the surface of the well. Incubate in a 37°C, 5% $CO_2$ incubator.

13. After 10 – 15 minutes, visually inspect the plate to ensure that newly thawed cell aggregates are evenly distributed across the surface of the wells. Plates that have not been properly agitated may have cell clumps aggregating toward the center of the wells. This uneven distribution at the center may later cause spontaneous differentiation of human ES/iPS cells. In the event clumps are not evenly distributed, agitate the plate gently from side to side and forward and backwards for a longer extended time.

14. The next day, replace with 3 mL per well of fresh PluriSTEM™ medium. Monitor & exchange medium daily.

15. Cultures should be fed with 4 mL PluriSTEM™ per well on Friday to ensure sufficient medium to sustain the cells over the weekend. Medium exchanges during the weekend are not necessary.

16. When pluripotent cultures have been maintained in PluriSTEM™ for at least 3 passages, media exchanges may be transitioned to every other day.

**RELATED PRODUCTS**

| cat # | description |
|---|---|
| SCM130 | PluriSTEM™ Human ES/iPS Medium |
| SCM134 | PluriSTEM™ Freeze Medium |
| SCR001 | ES Cell Characterization Kit |
| SCR002 | ES Cell Marker Sample Kit |
| SCR078 | Fluorescent Human ES/iPS Cell Characterization Kit |
| FCSC100107 | FlowCellect™ Human iPS Cell Characterization Kit |
| SCR004 | Alkaline Phosphatase Detection Kit |
| SCR545 | Human STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit |

**Please visit www.millipore.com for additional product information, test data and references**
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