BI
Biological Industries

CryoStem

A chemically defined, animal component-free, freezing medium, designed for the cryopreservation of hESC and hiPSC.

Cat. No.: 05-710-1D 10ml
05-710-1E 50ml
Store at: +2-8°C

Instructions for Use

Product Description

CryoStem™ is a novel animal-component free human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) freezing medium, that has been developed by Biological Industries.
CryoStem™ contains no serum, but rather methylcellulose and DMSO, hence eliminates the risk of contamination with potential adventitious agents, which often associated with the use of animal sera. Furthermore, it allows cells to be grown under a defined set of conditions. In general, when using serum-free media in cell culture, it is important to cryopreserve cells also in a medium free of serum.
After freezing with CryoStem™ and thawing, a very high percentage of viable hESC or hiPSC are obtained. In fact, comparative studies have shown that in most cases upon thawing and plating, higher viability and adhesion percentages are obtained in comparison to other freezing medium formulations including serum-containing freezing medium. Therefore, the use of CryoStem™ is also recommended for cell culture employing serum-supplemented growth media.

Features

- Chemically defined
- Sterile, 0.2µm membrane filtered. Manufactured under full aseptic conditions and practice following ISO 13408
- Animal components- free (ACF)
- Protein free
- For freezing human ES and iPS cells cultured in both feeder-free and feeder dependent conditions
- For cryopreservation of hPSC clumps or single cells
- High recovery efficiency: maintains excellent attachment ability as well as growth performance
- Maintains human ES and iPS cell pluripotency

Precaution and Disclaimer

1. For in vitro diagnostic use, and use as ancillary material in Cell- and Tissue-Based Therapies.
2. Do not use if a visible precipitate is observed in the freezing medium.
3. Do not use beyond the expiration date indicated on the product label.
4. Please refer to the Safety Data Sheet (SDS) for hazard information.

Instruction for use

Freezing human PSC :

Notes:

- hPSC may be freezed as clumps or single cells with high viability and minimal differentiation post thaw. The single cells hPSC can be thawed onto recombinant Laminin coated culture ware without the addition of Rock inhibitors. In case of using other matrices (e.g. Matrigel™) rock inhibitor is required.
- Keep Cryostem freezing medium on ice at all times.
- The procedure describes the cryopreservation of cells cultured in 6-well plate.

1. Aspirate hPSC culture medium.
2. Rinse wells with Dulbecco's PBS w/o Ca & Mg (Cat# 02-023-1), or DMEM:F-12 (1:1) (Cat# 01-170-1),
3. Add dissociation solution as desired. Cells may be detached using the enzyme and method that the culture has been routinely passaged with. In case of using collagenase, dispase or EDTA, incubate at 37°C or at room-temperature until the edges of the colonies begin to loosen from the plate. Incubation time will vary between cell lines, colony size and detachment solution used. Begin checking the culture after 3 minutes.
4. Cells cultured on laminin may be detached using Recombinant Trypsin-EDTA Solution (Cat# 03-079-1) to yield a single cell suspension.
5. Transfer the clumps or cell suspension to a conical tissue culture centrifuge tube.
6. Centrifuge at 200 x g for 5 minutes at room temperature. Remove and discard supernatant.
7. Gently suspend the pellet in ice cold CryoStem. The final volume is the number of vials desired multiplied by one ml In case of aggregates, do not break up cell clumps any more than necessary, two or three gentle pipeting motions are usually sufficient..
8. Quickly transfer 1 ml into each cryogenic vial.
9. Place the vials into a freezing container (e.g. Mr. Frosty™) and transfer to -80°C for overnight.
10. The following day, transfer vials to liquid nitrogen storage (vapor phase).

Thawing human ES or iPS cells:

1. Add 9ml of warm NutriStem® hPSC XF Medium into a conical tube.
2. Remove vial of Human Pluripotent Stem Cells from liquid nitrogen storage.
3. Immerse vial in a 37°C water bath, swirl gently.
4. When only an ice crystal remains, remove vial from water bath. Disinfect vial by rinsing with 70% ethanol.
5. In a sterile biological safety cabinet, transfer the contents of the cryogenic vial drop by drop to the 9ml culture medium in the previously prepared conical tube. Gently rock to continually mix the cells as the new cell drops are added to the tube.
6. Centrifuge cells at 200 x g for 5 minutes. Remove and discard supernatant.
7. Gently resuspend cell pellet in NutriStem® hPSC XF (with HSA) (Cat# 05-100-1) and plate as desired.
8. Refresh culture medium 48 hrs. after plating.

Quality Control

Each lot is tested on hPSC for colony recovery, morphology and differentiation after cryopreservation and thawing.

Auxiliary products

Product name	Cat. No.
Dulbecco's PBS (w/o Ca & Mg)	02-023-1
NutriStem® hPSC XF (with HSA)	05-100-1
AF NutriStem® hPSC XF (w/o HSA)	05-102-1
LaminStem™ 521	05-753-1
Recombinant Trypsin-EDTA Solution	03-079-1

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