CryoStem

Product Name: CryoStem
Catalogue number: 05-710-1D **Qty/Pk:** 10ml
Catalogue number: 05-710-1E **Qty/Pk:** 50ml

CryoStem is a chemically defined, animal component-free frezzing medium, designed for the cryopreservation of clumps of human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC)

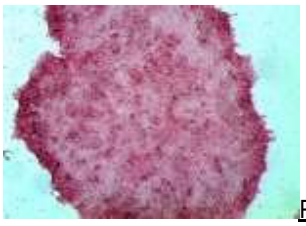
Features

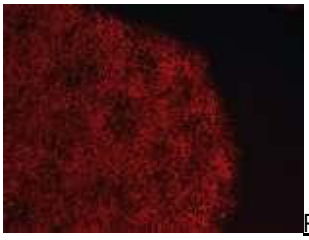
- **Fully defined: serum-, animal component-,and protein- free and chemically defined**
- **For freezing human ES and iPS cells cultured in both feeder-free (Matrigel) and feeder dependent (HFF, MEF) conditions.**
- **High Recovery efficiency: maintains excellent attachment ability as well as growth performance**
- **Maintains pluripotency of human ES and iPS cells**

Storage

Should be stored at 2-8ºC

Figure 1:
Alkaline Phosphatase (AP) staining of H1 cells at Passage 2 recovered from freezing condition in CryoStem freezing medium. Cells were maintained on feeder layer.
Cell colony exhibits a distinct morphology typical of pluripotent hESCs.

 Figure 2:
AP staining of H1 cells at Passage 2 recovered from freezing condition in CryoStem freezing medium.
Cells were maintained in feeder-free conditions.
Cell colony exhibits a distinct morphology typical of pluripotent hESCs.

 Figure 3:
H1 cell morphology and immunofluorescence analysis of hESC marker SSEA-4 at Passage 2, recovered from freezing condition in CryoStem freezing medium.

CryoStem PROTOCOL

A chemically defined, animal component-free, freezing medium, designed for the cryopreservation of clumps of human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC).

Cat.# 05-710-1D, Size: 10ml

Cat.# 05-710-1E, Size: 50ml

Store at: +2-8 °C

Precaution and Disclaimer:
For in vitro diagnostic use.
Do not use if a visible precipitate is observed in the freezing medium.
Do not use beyond the expiration date indicated on the product label.

Freezing hES Cells:
1. Aspirate hES cell culture media.
2. Rinse wells with Dulbecco's PBS w/o Ca & Mg (Cat# 02-023-1).
3. Add dissociation solution as desired, incubate till the colonies will begin to seperate.
To confirm colony separation from the plate, view plate under microscope.
4. Add NutriStem™ hESC XF with HSA (Cat# 05-100-1) and gently scrape the colonies off, transfer into conical tube.
5. Pellet cells by centrifuging at 200 x g for 5 minutes. Remove and discard supernatant.
6. Gently resuspend the pellet in cold CryoStem. Do not break up cell aggregates any more than necessary, two or three gentle pipeting motions are usually sufficient. (The final volume is the number of vials desired multiplied by one ml).
7. Transfer 1 ml into cryogenic vial.
8. Place cryovials in isopropanol freezing containers. Freeze overnight at -80°C.
9. The following day, transfer vials to liquid nitrogen storage.

Thaw hES Cells:
1. Remove vial of Human Embryonic Stem (hES) Cells from liquid nitrogen storage.
2. Immerse vial in a 37°C water bath, swirl gently.

3. When only an ice crystal remains, remove vial from water bath. Sterilize vial by rinsing with 75% ethanol.
4. Gently pipette cells into a 15ml conical tube with a 1ml or 5ml pipette.
5. Slowly add 9ml NutriStem™ hESC XF with HSA (Cat# 05-100-1) drop-wise to cells in the 15ml conical tube. While adding media, shake the tube gently to mix the hES cells.
6. Gently pipette cells up and down in the tube a few times. Avoid breaking up the cell aggregates too much.
7. Centrifuge cells at 200 x g for 5 minutes. Remove and discard supernatant.
8. Gently resuspend cell pellet in NutriStem™ hESC XF with HSA (Cat# 05-100-1) and plate as desired.
9. Refresh hES Cell Culture Media daily.

Quality Control
Each lot is tested on hES cells for colony recovery, morphology and differentiation after cryopreservation and thawing.

Auxiliary products

Product Name	Catalogue No.
Dulbecco's PBS (w/o Ca & Mg)	02-023-1
NutriStem™ hESC XF (with HAS)	05-100-1
AF NutriStem™ hESC XF (w/o HSA)	05-102-1