BI
Biological Industries

NutriStem® hESC XF

A defined, Xeno-Free (XF), Serum-Free (SF) Media, Designed to Support the Growth of Human Embryonic Stem Cells (hESC) and Induced Pluripotent Stem Cells (iPSC)

Cat. No.:	
05-100-1B 100ml	NutriStem® hESC XF, contains HSA
05-100-1A 500ml	Xeno-free medium for feeder-free and feeder-dependent culture of hESCs and hiPSCs
05-102-1B 100ml	AF NutriStem® hESC XF, without HSA
05-102-1A 500ml	Xeno-free medium for feeder-dependent culture of hESCs and hiPSCs

Instructions for Use

Product Description

Traditional human Embryonic Stem Cells (hESC) culture methods require the use of mouse or human fibroblast feeder layers, or feeder-conditioned medium. These culture methods are labor-intensive, hard to scale and it is difficult to maintain hESC's undifferentiated due to undefined conditions. NutriStem® hESC XF media were developed with a leading group in stem cell research, to enable the maintenance and expansion of hESCs with feeder cells or in feeder-independent culture. NutriStem® hESC XF support the culture of undifferentiated hESC in serum-free conditions without any animal components on mouse feeder cells (MEF), Matrigel or Human Foreskin Fibroblasts (HFF). The media contain recombinant human basic fibroblast growth factor (rh bFGF) and recombinant human transforming growth factor β (rh TGFβ). The media have been successfully tested and proven to maintain the pluripotential nature of hESC.
For long-term growth of hESC without feeder cells it is recommended to use NutriStem® hESC XF (with HSA).

Features

- Serum Free Media (SFM), xeno-free (XF): all components are defined and from non-animal origin including proteins.
- The proteins that are used are rh bFGF, rh TGFβ, human transferrin and recombinant human insulin.
- A complete, ready-to-use formulation (no additions are required).
- Contain Alanyl glutamine. Do not contain antibiotics.
- Enable culture of hESC's without feeder cells.
- Can be used with mouse or human feeder cells, Matrigel and with matrix from human cells.
- Intended for use with 5% CO_2 (ordinary conditions).
- Support long–term growth of hESC, maintaining the ability of hESC to differentiate, without any signs of karyotype abnormalities.

Precautions and Disclaimer

1. For in vitro diagnostic and research use only.
2. Do not use if a visible precipitate is observed in the medium.
3. Do not use NutriStem® hESC XF media beyond the expiration date indicated on the product label.

Storage and Stability

NutriStem® hESC XF should be stored at -20˚C. Thaw the medium overnight at 2-8°C, protected from light. Upon thawing, the medium may be stored at 2-8˚C for 2 weeks. Dispense into aliquots to avoid repeated freezing and thawing. Protect the medium from light.
Shelf Life: Refer to product label for expiration date.

Instructions for Use

For complete instructions on how to maintain hESC's in NutriStem® hESC XF, see technical manual guide.
Specific ES cell culture protocol may require optimization for best results. The following protocol is a generic guideline.
When using AF NutriStem® (05-102-1) for feeder-independent culture, add 5% of Bio-Pure HSA (05-720-1) to the medium (0.5% final).

Protocol for maintenance of undifferentiated hESC:

1. Preparation of feeder cells:

1.1 0.1% Gelatin Coating of Plates:

Cover plating dish according to the following table:

Plate/ dish	Volume of gelatin per well
4 wells	0.5 ml
6 wells	2 ml
35mm	2 ml
10cm^2	10 ml

Leave at room temperature or in incubator for at least two hours.

Note: It is highly recommended to prepare gelatin-coated plates 24 hour before use.

1.2 Preparation of MEF feeder cells:

1.2.1 Add 8µg/ml mitomycin C into a culture flask and incubate for two hours.
1.2.2 Wash four times with D-PBS.
1.2.3 Add 2ml of trypsin/EDTA and cover the entire culture-flask surface.
1.2.4 Incubate for 6 minutes.
1.2.5 Tap side of the flask to loosen the cells. Add 4ml of culture medium to neutralize the trypsin.
1.2.6 Remove cell suspension into conical tube.
1.2.7 Centrifuge for five minutes at 2000rpm.
1.2.8 Remove suspension, re-suspend in 10ml of culture medium and pipette in order to re-suspend the pellet.
1.2.9 Count cells and resuspend in desired medium volume.
1.2.10 Add cell suspension into gelatin pre-coated culture dishes. We recommend 4x10^5 cells per well in six-well plates.
1.2.11 Allow to set for at least two hours before plating hES cells.

Notes:

1. MEF concentration can also be calculate as 3x10^4 cells per cm^2.
2. Do not use NutriStem® hESC for MEF-covered plate preparation. Prepare MEF-covered plate using recommended culture medium and change the medium before plating hES cells.
3. Plates may be used within two weeks of preparation.

1.3. Preparation of HFF feeder cells:

1.3.1 Add 8µg/ml mitimycin C into culture flask and incubate for two hours.
1.3.2 Wash four times with D-PBS.
1.3.3 Add 2ml of trypsin-EDTA and cover the entire culture-flask surface.
1.3.4 Incubate for 6 minutes.
1.3.5 Tap side of the flask to loosen cells. Add 4ml of culture medium to neutralize the trypsin.
1.3.6 Remove cell suspension into conical tube.
1.3.7 Centrifuge for five minutes at 1500rpm.
1.3.8 Add 10ml of culture medium and pipette up and down in order to detach the cells.
1.3.9 Count cells and re-suspend in desired medium volume.
1.3.10 Add cell suspension into gelatin pre-coated culture dishes. We recommend 4x105 cells per well in six-well plates.
1.3.11 Allow to set for at least five hours before plating hES cells.

Notes:

1. HFF concentration can be calculate as 3x10^4 cells per cm^2 .
2. If possible, allow plate to set overnight before plating hES cells.

2. hES cells Splitting Dissociation medium:

1mg/ml collagenase in DMEM/F12 (1:1) (Cat. No. 01-170-1)
Splitting protocol:

2.1 Remove medium from well. Add 0.5ml dissociation medium, and incubate for at least 30 minutes.
2.2 Add 1ml of culture medium and gently detach cells with 5ml pipette.
2.3 The MEF feeder layer will remain on the plate.
2.4 Collect cell suspension and put into conical tube.
2.5 Centrifuge 3 minutes at 800rpm at 4°C.
2.6 Re-suspend cells in medium and plate on feeder covered plate.

Notes:

1. For effective separation of hES cells from the feeder cells, longer collagenation is recommended.
2. hES cells may be incubated in collagenase for up to three hours.

3. Preparation of Matrigel-coated plates:

3.1 Dilute the Matrigel 1:40 in cold NutriStem® hESC XF.
3.2 Add the diluted Matrigel to cell culture dishes as described in the Matrigel protocol.
3.3 Incubate for 40 minutes.
3.4 Collect the remaining Matrigel.
3.5 Seed hES cells in the Matrigel pre-coated plates.

Adaptation of hESC's to NutriStem® hESC XF

Cells can be transferred directly to NutriStem® hESC XF, without prior adaptation.

Quality Control

NutriStem® hESC XF performance is tested for optimal maintenance and expansion of undifferentiated hESC. Additional standard evalutions are pH, osmolality, endotoxins and sterility tests.

Auxiliary Products

Product	Cat. No.
Dulbecco's Phosphate Buffered Saline w/o Calcium and Magnesium (D-PBS)	02-023-1
0.1% Gelatin Solution	01-944-1
Trypsin-EDTA Solution B (EDTA 0.05%, Trypsin 0.25%) with Phenol Red	03-052-1
Bio-Pure Human Serum Albumin (HSA), 10% solution	05-720-1
CryoStem	05-710-1

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