BI
Biological Industries

NutriStem® hPSC 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 05-100-1A 500ml	NutriStem® hPSC XF, contains HSA Xeno-free medium for feeder-free and feeder-dependent culture of hPSC and hiPSC
05-102-1B 100ml 05-102-1A 500ml	AF NutriStem® hPSC XF, without HSA Xeno-free medium for feeder-dependent culture of hPSC and hiPSC

Instructions for Use

Product Description

Traditional human Embryonic Stem Cells (hESC) and human induced Pluripotent Stem Cells (hiPSC) 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 hPSC pluripotency due to undefined conditions.

NutriStem® hPSC XF media were developed with a leading group in stem cell research, to enable the maintenance and expansion of hESC and hiPSC in feeder-free culture or with feeder cells.

NutriStem® hPSC XF support the culture of undifferentiated hESC and hiPSC in serum-free conditions without any xeno-derived components on feeders such as Mouse Embryo Fibroblasts (MEF) and Human Foreskin Fibroblasts (HFF) as well as with Matrigel™ and recombinant laminin. The media contain recombinant human basic fibroblast growth factor (rh bFGF) and recombinant human transforming growth factor (rh TGFβ). The media have been successfully tested and proven to maintain the pluripotent nature of hESC and hiPSC.

For long-term growth of hESC and hiPSC without feeder cells it is recommended to use NutriStem® hPSC XF with HSA (BI Cat.# 05-100-1).

Features

- Serum Free Media (SFM), xeno-free (XF): all components are defined and xeno-free including proteins.
- Sterile, 0.1µm membrane filtered, under full aseptic conditions and practice following ISO 13408
- The proteins used: HSA (Human Serum Albumin), 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 and hiPSC without feeder cells.
- Can be used with mouse or human feeder cells, Matrigel™, laminin and with matrix from human cells.
- Can be used with laminin 521 (e.g. LaminStem™ 521, BI Cat.# 05-753-) using single cell passage.
- Intended for use with 5% CO_2 (ordinary conditions).
- Support long–term growth of hESC and hiPSC, and maintain their ability to differentiate, without any signs of karyotype abnormalities.

Precautions 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 medium.
3. Do not use NutriStem® hPSC XF and AF NutriStem® hPSC XF media beyond the expiration date indicated on the product label.

Storage and Stability

NutriStem® hPSC XF should be stored at -20°C. Upon thawing at room-temperature or at 2-8°C, the medium may be stored at 2-8°C for 2 weeks. In case smaller quantities are needed, use aseptic techniques to dispense into aliquots so repeated freezing and thawing are avoided. NutriStem® hPSC XF must be warmed to room temperature (15°C-30°C) before use. To insure stability of the medium, warm only the amount needed. 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 and hiPSC in NutriStem® hPSC XF, see technical manual guide.
Specific pluripotent cell culture protocol may require optimization for best results. The following protocol is a generic guideline.
When using AF NutriStem® XF (05-102-1) for feeder-independent culture, add 5% of Bio-Pure Human Serum Albumin (HSA), 10% solution (BI Cat.# 05-720-1) to the medium (0.5% w/v final).
Use aseptic techniques.

Protocol for maintenance of undifferentiated hESC and hiPSC:

1. Feeder-dependent culture

Gelatin coating:

Coat cultureware using 0.1% Gelatin solution according to the following table:

Plate/ dish	Volume of gelatin per well
4/24 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.

Preparation of feeder cells (Mitomycin C treated):

1. MEF feeder cells should be passaged into T75 flasks twice and used on the third passage.
2. Discard the medium and add 8µg/ml mitomycin C (6-7ml) into a culture flask and incubate for two hours.
3. Wash three times with D-PBS without calcium and magnesium (02-023-1).
4. Add 3ml of trypsin-EDTA solution and cover the entire culture-flask surface.
5. Incubate for 6 minutes.
6. Tap side of the flask to loosen the cells. Add 10ml of feeder culture medium to neutralize the trypsin.
7. Aspirate cell suspension into conical tube.
8. Centrifuge for five minutes at 2000rpm.
9. Remove supernatant, add feeder culture medium and pipette in order to re-suspend the pellet.
10. Count cells and re-suspend in desired medium volume.
11. Add cell suspension into gelatin pre-coated culture dishes. We recommend 3.5x10^5 cells per well in six-well plate (2ml/well).
12. Incubate the feeder cells for at least two hours before plating the hPSC (cells must be spreaded).
13. When platting hPSC, change the feeder medium to NutriStem® hPSC XF medium.

Notes:
- Feeder concentration can also be calculated as 3.5x10^4 cells per cm^2.
- Do not use NutriStem® hPSC for MEF culture. Prepare MEF-covered plate using recommended culture medium and change the medium before plating the cells.
- Plates may be used within 10 days of preparation.
- It is highly recommended to prepare MEF plates 24 hours before use.

Passage of hPSC:

Collagenase solution:
Dissolve Collagenase type IV in DMEM/F-12 (1:1) medium (BI Cat.# 01-170-1) to a concentration of 1mg/ml and filter sterilize with a 0.22µm pore size filter unit. Collagenase solution can be stored at 2-8oC for up to 2 weeks.

Protocol:
1. Remove medium from well. Add 1.0ml collagenase solution.
2. Incubate at 37°C or at room temperature until the edges of the cell colonies begin to loosen from the plate.
   Note: Incubation times will vary between cell lines and colony sizes. Begin checking the culture after 3 minutes. Do not over-incubate the culture, as hPSC are sensitive to enzymatic stress and may lift from the plate during the wash step.
3. Aspirate the Dissociation Solution and wash the cells twice with 2ml of sterile DMEM/F-12 (1:1).
4. Add 1ml of culture medium and gently wash off and detach hPSC using 5ml pipette. Most of the feeder layer cells will remain on the plate.
5. Collect cell suspension and put into conical tube.
6. Centrifuge 3 minutes at 800rpm at room temperature.
7. Re-suspend hPSC using NutriStem® hPSC XF medium by gently pipetting up and down with a 5ml pipet to break hPSC clumps and plate in feeder-covered plate with 2.5-3ml NutriStem® hPSC XF.
8. Change the medium daily with 2.5-3.0 ml/well fresh NutriStem® hPSC XF until the hPSC colonies are large enough to passage.
   Note: For effective separation of hPSC from the feeder cells, longer dissociation time is recommended (up to 1hr.).

2. Feeder-free culture

Matrigel™ based culture

Matrigel™ preparation

Matrigel™ should be thawed on ice, aliquoted and refrozen. For full instructions and recommendations on aliquot size, please refer to the insert supplied with the product.

Preparation of Matrigel™ Aliquots:

1. Thaw Matrigel™ on ice at 2-8°C for overnight to avoid the formation of a gel.
2. Dilute Matrigel™ 1:1 with cold DMEM: F12 (1:1) medium (BI Cat.# 01-170-1). Mix well with a cold pipette.
3. Keep mixture on ice.
4. Aliquot into pre-chilled 15ml tubes (for aliquot stability, please refer to the insert supplied with the product). Store at -70°C.

Coating Plates with Matrigel™

1. Slowly thaw Matrigel™ aliquot on ice at 2-8°C, to avoid the formation of a gel.
2. Dilute the Matrigel™ aliquot 1:20 in cold DMEM: F12 (1:1).
3. Add 1ml of Matrigel™ solution to each well of 6-well plate.
4. Incubate the plate for at least 1-2 hours at room temperature or overnight at 2-8°C. Plate with Matrigel™ solution can be parafilm® sealed and stored at 2-8°C for one week. Do not use the plate if the Matrigel™ solution does not completely cover the surface of the wells.
5. Before use, warm to room temperature, remove Matrigel™ solution and immediately cover with NutriStem® hPSC XF.

Enzymatic passaging of hPSC grown in NutriStem® hPSC XF on Matrigel™

We recommend using Collagenase IV at 1mg/ml. If using other enzyme-based dissociation solutions (Dispase or recombinant trypsin), optimal conditions should be determined by the user.

Protocol:

1. Aspirate the culture medium from the well.
2. Wash the well once with warm DMEM: F12 (1:1) medium (BI Cat.# 01-170-1).
3. Add 1ml per well of warmed Collagenase IV (1mg/ml).
4. Return the plate to the 37°C 5% CO_2 incubator. Check the culture every 5-10 minutes. Do not over-expose the cells to Collagenase IV. Observe the colonies under the microscope. Incubate until the edges of the colonies begin to curl up but the colonies should remain attached to the plate surface.
5. Aspirate the Collagenase solution and wash very gently at least once with DMEM: F12 (1:1) medium (BI Cat.# 01-170-1).
6. Add 2ml of pre-warmed NutriStem® hPSC XF medium to the well.
7. Gently scrape and wash the colonies off with 5ml glass pipette.
8. Repeat the scraping and pipetting action 3-4 times until all the colonies have been removed from the well surface. Pipette gently to avoid breaking up the colonies into too small clumps.
9. Transfer the detached colonies into a sterile conical tube.
10. Use another 2ml of medium to wash the well. Transfer the cell clumps into the same tube.
    Note: The split ratio of NutriStem® hPSC XF medium-based culture is usually 1:6-1:8 every 3-5 days.
11. To obtain the desirable split ratio, increase the volume of the clumps suspension in the tube with NutriStem® hPSC XF medium accordingly.
12. Prepare a new Matrigel™-coated 6-well plate by washing once with DMEM: F12 (1:1). Gently add 3ml NutriStem® hPSC XF medium to each well to be seeded.
13. Mix the hPSC clumps suspension in the tube to break cell clumps by gently pipetting up and down with a 5ml pipet.
14. Gently and evenly add 1ml of the suspended hPSC clumps to each new well to be seeded.
15. Place the plate in the 37°C 5% CO_2 incubator and carefully move the plate back and forth and side to side to evenly distribute the clumps throughout the well.
16. Allow the colonies to attach in 37°C 5% CO_2 incubator.
17. After 48 hours, change the medium daily with 2.5-3.0 ml/well fresh NutriStem® hPSC XF until the hPSC colonies are large enough to passage.
18. It is possible to perform higher volume feed (4-5ml) on the weekend and the next medium change two days later.
19. If the culture is at optimal density, the cells can be split every 3-4 days using a 1:6-1:8 splitting ratio (colonies from 1 well of a 6-well plate can be plated in 6-8 new wells of a 6-well plate). If the colonies are too dense or too sparse, adjust the splitting ratio accordingly.

Enzyme-free passaging of hPSC grown in NutriStem® hPSC XF on Matrigel™

Dissociation solution:

0.5mM EDTA in DPBS: prepare 0.5mM EDTA by combining 50 µL of 0.5M EDTA (BI Cat.# 01-862-1), pH 8.0 with 50ml of DPBS without calcium and magnesium (BI Cat.# 02-023-1). Filter sterilize the solution and store at room temperature.

Protocol:

This procedure describes the passage of the colonies as very small clumps. Volumes are for 1 well of 6-well plate.

1. Wash cells twice with 2ml DPBS without calcium and magnesium (BI Cat.# 02-023-1). Add 1ml 0.5mM EDTA solution, swirl the vessel to coat the entire cell surface and quickly discard.
   Note: Do not expose cells to the EDTA solution for more than needed for a quick wash at this point.
2. Add 1ml of 0.5mM EDTA solution and incubate for 3-4 minutes at room temperature.
   Do not move the plate during EDTA exposure.
3. Gently remove the EDTA solution and add carefully 1ml NutriStem® hPSC XF medium.
4. Detach and break colonies by gently pipetting up and down 3-4 times with a 1ml tip. Make sure the pipetting washes the entire well.
5. Plate the cell aggregates at the desired density in Matrigel™ coated wells with 4ml pre-warmed NutriStem® hPSC XF medium. Usually a splitting ratio of 1:8-1:10 every 4 days is required.
6. Place the plate in a 37°C 5% CO_2 incubator. Move the plate several times back and forth and side to side motions to distribute the aggregates evenly in the well.

7. After 48 hours, change the medium daily with 2.5-3.0ml/well fresh NutriStem® hPSC XF until the hPSC colonies are large enough to passage.
8. It is possible to perform higher volume feed (4-5ml) on the weekend and the next medium change two days later.
   Note: Do not move the plate during the first 48 hours post-split (this may increase differentiation of hPSC).

Laminin based culture

LN521 coating procedure using 0.5-1µg/cm^2 in a 6-well plate. For a detailed protocol, follow laminin manufacturer instructions.

General coating protocol: (0.5µg/cm^2)

1. Slowly thaw recombinant laminin (LN521) at 2-8°C.
2. Thawed laminin stock may be stored at 2-8°C under aseptic conditions.
3. Dilute 300µl LN521 (0.1mg/ml) with 12ml DPBS **with** calcium and magnesium, (50µl/2ml/well).
4. Add 2ml/well of the diluted LN521.
5. Seal the plate with plastic film (e.g., Parafilm®) to prevent evaporation, and incubate overnight at 2-8°C. Make sure the laminin solution is evenly spreaded across the surface. Note that the laminin matrix will be inactivated if dried.

Notes:

- Optimal coating concentration is cell-dependent and should be calibrated. 1 µg/cm^2 should work well for most hPSC.
- Coated plates may be stored aseptically at 2-8°C.
- Rapid coating may be done at 37°C for 2 hours.

Single cell passage of hPSC cultured on LN521 (enzymatic procedure, volumes are for 1 well of 6-well plate)

1. Wash cells twice with 2ml/well DPBS without calcium and magnesium
2. Add 1ml of recombinant Trypsin-EDTA Solution (BI cat.# 03-079-1) and incubate at 37°C for 2-4 minutes. Exposure time may vary and should be adjusted.
   Note: Do not over-expose the cells to the recombinant Trypsin-EDTA Solution.
3. Detach the cells by gently pipetting up and down. Add 4ml 1xSBTI (BI Cat.# 03-048-1) to the recombinant Trypsin-EDTA Solution.
4. Detach and break colonies by pipetting 5-6 times up and down with a 1ml pipette. Make sure the pipetting washes the entire well.
5. Collect cells into new sterile tube.
6. Centrifuge at 200g for 5 minutes.
7. Suspend the pellet with 1 ml of fresh medium.
8. Count cells.
9. Plate cells at the desired density in LN521 pre-coated wells with 3-4 ml pre-equilibrated medium. Usually, 10-20,000 /cm^2 for splitting every 4-5 days.
10. Place the plate in a 37°C 5% CO_2 incubator. Move the plate several times in back and forth and side to side motions to evenly distribute the cells in the well.
11. Perform daily medium change after 48 hours with 2.5-3.0ml/well fresh NutriStem® hPSC XF until the hPSC colonies are large enough to passage. Do not move the plate during the first 48 hours post-split.
12. It is possible to perform higher volume feed (4-5ml) for the weekend and skip 2 days without changing the medium. In this case, the cells must be passaged at the end of the week.

Notes:

- It is critical not to move the seeded plates during the first 48 hours after splitting as this may increase differentiation of hPSC.
- Cells are ready to be passaged when cell culture is ≥60% confluent. Optimal seeding densities will vary from one cell line to another and must be determined empirically for your system. With optimal culture conditions and seeding density, most cell lines will reach confluency within 4-6 days and expand 10-25 folds.

Auxiliary Products

Product	Cat#
DPBS, no calcium, no magnesium	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
LaminStem™ 521	05-753-1
Recombinant Trypsin-EDTA solution	03-079-1
DPBS with Ca & Mg	02-020-1
SBTI Solution (50x)	03-048-1
EDTA 0.5M Solution	01-862-1

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