BI
Biological Industries

NutriStem® V9 XF Medium

A defined, xeno-free (XF), serum-free (SF) culture medium for hPSC using vitronectin

Instructions for Use

Product Description

NutriStem® V9 XF medium is a defined, xeno-free, serum-free medium designed to support the growth and expansion of human pluripotent stem cells (hPSC) using vitronectin and enzyme-free passage with EDTA. It contains only the essential components required for long-term maintenance of hES and hiPS cells. NutriStem® V9 XF medium shows superior proliferation rates during long-term cultures, while maintaining the pluripotency of the cells. NutriStem® V9 XF medium allows culture of hPSC in vitronectin pre-coated culture, as well as direct addition of Vitronectin ACF (animal component-free) to the medium (no need for pre-coating).

Ordering Information

Product Description	Cat. No.	Size
NutriStem® V9 XF Basal Medium	05-105-1A	500 ml
NutriStem® V9 XF Supplement Mix	05-106-1F	1 ml
Vitronectin ACF	05-754-0002	200 µg
0.5M EDTA Solution	01-862-1B	100 ml

Features

- Maintains human ES and iPS cell pluripotency throughout long-term culture
- Superior results using vitronectin, may be used with Matrigel coated plates
- Precoating- free option: plating cells without first pre-coating with vitronectin
- Defined, serum-free, xeno-free
- Produced under cGMP guidelines
- Weekend free feeding regime
- Cytokine-free basal medium, applicable for EBs formation, reprogramming, and differentiation
- The proteins used: HSA (Human Serum Albumin), rh bFGF, rh TGF β, human transferrin and recombinant human insulin
- Contains alanyl glutamine. Does not contain antibiotics

Storage and Stability

NutriStem® V9 XF basal medium and NutriStem® V9 XF Supplement Mix should be stored at (-10) °C to (-20)°C.

Precaution and Disclaimer

1. Do not use if a visible precipitate is observed.
2. Do not use beyond the expiration date indicated on the product label.
3. Please refer to the Safety Data Sheet (SDS) for hazard information.

Quality Control

Each lot is tested for performance using H1 hESC by expansion of the cells for 3 passages, immunofluorescence staining for pluripotency marker, and morphology. Other tests include sterility, pH, osmolality and endotoxins levels.

Instructios for Use

Preparation of complete NutriStem® V9 XF medium

1. Thaw NutriStem® V9 XF basal medium at 2-8°C or at room temperature, and NutriStem® V9 XF Supplement Mix on ice. Do not thaw the frozen supplement at 37°C.
2. Add 1ml NutriStem® V9 XF supplement mix to 500ml basal medium and swirl the bottle to mix. Protect the medium from light.
3. Use complete NutriStem® V9 XF medium within 2 weeks when stored at 2-8°C.
4. Before use, NutriStem® V9 XF must be warmed to room temperature (15 -30°C). To ensure stability of the medium, warm only the amount needed.
5. For long-term storage, prepare working volume aliquots of complete NutriStem® V9 XF medium and store at (-10) °C to (-20)°C for up to 6 months. Thaw aliquots of complete NutriStem® V9 XF medium overnight at 2-8°C. Once thawed, keep aliquots at 2-8°C and use within 2 weeks.

Vitronectin ACF Coating

Preparation of Vitronectin ACF 0.5mg/ml

Best results obtained with Vitronectin ACF (animal component-free) (BI Cat# 05-754-0002). When pre-coating plates, vitronectin from other suppliers may also be used.

Vitronectin ACF is a lyophilized protein and should be stored at (-10) °C to (-20)°C up to expiration date.

Reconstitution procedure should be performed on ice.

1. Spin down the vial before reconstitution.
2. To the original 0.2mg vial add 0.4ml of sterile tissue culture water. DO NOT VORTEX.
3. Incubate on ice for 2-5 minutes.
4. Gently mix by pipetting up and down.
5. Keep on ice for immediate use (up to 1 week at 2-8°C)
6. For long-term storage aliquot and freeze at (-80)°C. Additional freeze-thaw cycles are not recommended.

Note: Take into account that to coat 6x10cm^2 (6-wells of a 6-well plate), 60µl of the 0.5mg/ml is required.

Preparation of pre-coated culture dishes with Vitronectin ACF

This procedure is for the pre-coating of a 10cm^2/well (1 well in a 6-well tissue culture plate), at a concentration of 0.5µg/cm^2.

Optimal coating concentration is cell-dependent and should be calibrated. 0.5-1µg/cm^2 should work well for most hPSC lines.

1. Thaw Vitronectin ACF on ice.
2. Dilute 10µl of Vitronectin ACF 0.5 mg/ml in 2ml DPBS without calcium and magnesium (BI Cat# 02-023-1).
3. Add 2ml diluted Vitronectin ACF solution to 1-well of tissue culture treated 6-well plate. Use table below for other culture ware.
4. Incubate at room temperature for 1 hour.
   Note:
   Coated plates may be stored aseptically at 2-8°C for up to 7 days. Seal the plate with laboratory film (e.g., Parafilm®) to prevent evaporation, and store. Do not allow the plate to dry.
   Prior to use, pre-warm the culture vessel to room temperature, wash the plate once with DPBS without calcium and magnesium, and add 3 ml/well of complete NutriStem® V9 XF medium.
5. For immediate use, aspirate and discard Vitronectin ACF solution. Do not allow the plate to dry.
6. Immediately wash the culture plate once with 2ml of DPBS without calcium and magnesium.
7. Add 3ml of complete NutriStem® V9 XF medium.

Recommended volume for Vitronectin ACF coating procedure

Culture ware	24 well	12 well	6-well / 35mm ware
Surface area (cm^2)	2	4	10
Volume of Vitronectin ACF 0.5mg/ml for coating at 0.5µg/cm^2	2µl	4µl	10µl
Volume of DPBS without calcium and magnesium	0.4ml	0.8ml	2ml

Precoating- free culture

A friendly-to-use procedure in which Vitronectin ACF is added directly into pre-equilibrated NutriStem® V9 XF medium prior to hPSC seeding.

The precoating-free procedure will require 0.25-0.375µg/cm^2 of Vitronectin ACF. Optimal coating concentration is cell-dependent and should be calibrated; it is recommended to test 25-50% less Vitronectin ACF than the concentration used in the standard pre-coating procedure.

Example

Coating concentrations and Vitronectin ACF volume required to coat 10cm² (1 well in a 6-well tissue culture plate)

Precoating procedure	Vitronectin ACF coating concentration	0.5µg/cm²
	Vitronectin ACF volume required for pre-coating procedure *	10µl
Precoating-free procedure	Vitronectin ACF coating concentration	0.25-0.375µg/cm²
	Vitronectin ACF volume added to 3ml medium *	5-7.5µl

* From 0.5mg/ml solution

Precoating-free protocol

This procedure is for the culture of hPSC in 10cm² (1 well in a 6-well tissue culture plate).

1. Thaw Vitronectin ACF on ice.
2. Add 3ml/well of complete NutriStem® V9 XF medium.
3. Equilibrate for at least 30 minutes in a 37°C CO_2 incubator.
4. Harvest cells according to the procedure described below.
5. Add 5-7.5µl of Vitronectin ACF to the 3ml pre-equilibrated NutriStem® V9 XF medium and swirl.

   Note:
   Vitronectin ACF may also be added before NutriStem® V9 XF pre-equilibration.
6. Perform enzyme-free cell cell passage according to the procedure described below.
7. Plate the cell aggregates at the desired density in the 3ml complete NutriStem® V9 XF medium containing the Vitronectin ACF. Usually a splitting ratio of 1:8-1:20 every 4 days is required.
8. Place the plate in a 37°C CO_2 incubator. Move the plate several times back and forth, and side to side to distribute the aggregates evenly in the well.
9. After 48 hours, change the medium daily with 3ml/well complete NutriStem® V9 XF medium until the colonies are large enough to passage.

Enzyme-free passaging of hPSC on Vitronectin ACF

Established culture is usually passaged every 4-5 days. Culture density is a critical aspect for maintaining healthy undifferentiated hPSC in NutriStem® V9 XF medium. Sub-optimal confluency may lead to differentiation. Passage time should be determined by colony size and density, NOT by the number of colonies in the well.

The culture should be passaged when one or more of the following occur:

- hPSC colonies become too dense
- hPSC colonies become too large
- The colonies cover around 80% of the surface area of the culture vessel
- Differentiation rate is higher than 15%

If the colonies are too dense or too sparse, adjust the splitting ratio accordingly

If the hPSC culture reaches confluency, splitting should be performed within the next 24 hours

Preparations prior to passage procedure

- 0.5mM EDTA dissociation solution:
  Prepare 0.5mM EDTA by combining 50µL 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). If required, filter the solution for sterility and store at room temperature for up to 6 months.
- Vitronectin ACF precoated plate: wash once with DPBS without calcium and magnesium, add 3ml complete NutriStem® V9 XF medium and pre-equilibrate in a CO_2 incubator for 30 minutes.

Enzyme-free passage protocol

This procedure describes the passage of hPSC colonies as very small aggregates. Volumes are for 10cm²/well (1 well of 6-well plate).

1. Wash cells twice with 2ml DPBS without calcium and magnesium (BI Cat# 02-023-1).
2. Add 1ml 0.5mM EDTA solution, swirl the plate to coat the entire cell surface and quickly aspirate and discard .
   Note: Do not expose cells to the EDTA solution for more than needed for a quick wash at this point.
3. Add 1ml of 0.5mM EDTA solution and incubate for 4-5 minutes at room temperature or 37°C.
   Note: Incubation time and temperature may vary between cell lines. For delicate hPSC lines, 3-4 minutes at **room temperature** are sufficient.
4. Gently remove the EDTA solution and carefully add 1ml complete NutriStem® V9 XF medium.
5. Gently pipetting up and down 3-4 times with a 1ml tip to detach and break colonies. Make sure the pipetting washes the entire well.
   Note: Some colonies may not be fully detached by pipetting 3 to 4 times. Do not scrape them or apply additional pipetations since each pipetation reduces aggregate size up to single cell suspension.
6. Plate the cell aggregates at the desired density in Vitronectin ACF pre-coated wells with 3ml pre-equilibrated complete NutriStem® V9 XF medium. Usually a splitting ratio of 1:8-1:20 every 4 days is required.

7. Place the plate in a 37°C CO_2 incubator. Move the plate several times back and forth, and side to side to distribute the aggregates evenly in the well.
8. After 48 hours, change the medium daily with 3ml/well complete NutriStem® V9 XF medium until the colonies are large enough to passage.

Notes:
- Do not move the plate during the first 48 hours post-split (this may increase differentiation of hPSC).
- It is possible to perform a higher volume feed (5-6ml) for the weekend and skip 2 days without changing the medium.

Cryopreservation of hPSC using CryoStem™ Freezing Medium

The following procedure uses CryoStem Freezing Medium (BI Cat. No. 05-710-1). The protocol below is based on freezing hPSC as aggregates with EDTA-based dissociation from a 6-well plate. For best results, the cells should be healthy, high-quality, and showing minimal differentiation (less than 10% in culture.) Freezing should take place when cells are actively growing, reaching approximately 60 to 70% confluency in culture.

Notes:
- The typical density for freezing hPSC from an EDTA-based passage is one well of cells (from a 6-well plate) per 2-4 cryogenic vials (freezing volume: 1ml of CryoStem™ Freezing Medium per vial).
- hPSC may be stored frozen as small aggregates and do not require the addition of ROCK inhibitors.
- Keep CryoStem™ Freezing Medium on ice at all times.

Protocol

The procedure describes the cryopreservation of cells cultured in 10cm² (1 well in 6-well plate).

1. Wash cells twice with 2ml DPBS without calcium and magnesium (BI Cat# 02-023-1).
2. 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.
3. Add 1ml of 0.5mM EDTA solution and incubate for 4-5 minutes at room temperature or 37°C.
   Note: Incubation time and temperature may vary between cell lines. For delicate hPSC lines, 3-4 minutes at **room temperature** are sufficient.
4. Gently remove the EDTA solution and carefully add 1ml complete NutriStem® V9 XF medium.
5. 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.
6. Transfer the clumps suspension to a conical tissue culture centrifuge tube.
7. Centrifuge at 200xg for 5 minutes at room temperature.
8. Remove and discard supernatant being careful not to disturb the loosely-packed colony pellet. Tap the conical vial to loosen the cell pellet.
9. Gently suspend the pellet with ice-cold CryoStem™ Freezing Medium. The final volume is the number of vials desired multiplied by 1ml. Make sure not to break up cell clumps any more than necessary, two gentle pipetting motions are usually sufficient.
10. Quickly but gently add 1ml of the cell suspension to each cryogenic vial.
11. Transfer the vials into a freezing container (e.g., Mr. Frosty), and place in a -80°C freezer or use controlled-rate freezing equipment.
12. The following day transfer the frozen vials to the vapor phase of a liquid nitrogen storage tank for long-term storage.

Thawing hPSC using NutriStem® V9 XF Medium

Prior to thawing hPSC, be sure to have a 6-well plate coated with Vitronectin ACF.

hPSC are usually cryopreserved from one 60 to 70% confluent well of 6-well plate to 2-4 cryogenic vials, intended to be thawed into 1 well of 6-well plate.

Protocol

Prepare a new coated plate:
1. Label the plate with the appropriate hPSC information: cell line, passage number, and thawing date.
2. Wash the wells; aspirate Vitronectin ACF solution from the wells and add 2ml per well DPBS without calcium and magnesium (BI Cat# 02-023-1). Gently swirl the medium around the well, aspirate and discard.
3. Immediately add 3ml of complete NutriStem® V9 XF medium.
4. Equilibrate for at least 30 minutes in a 37°C CO_2 incubator.

Thaw:
1. Add 9ml of warm complete NutriStem® V9 XF medium into conical tube.
2. Remove a cryogenic vial of hPSC from the liquid nitrogen storage tank.
3. Place the vial in a 37°C water bath; do not submerge the cap of the vial in the water as this could contaminate the cells. When only a small ice crystal remains, spray the entire vial with 70% ethanol for disinfection, and wipe.
4. In a sterile biological safety cabinet, transfer the content of the cryogenic vial drop by drop to the 9ml NutriStem® V9 XF medium in the conical tube.
5. Gently rock to continually mix the cells as the new cell drops are added to the tube.
6. Centrifuge the cells at 200xg for 5 minutes.
7. Carefully aspirate supernatant. Be careful not to aspirate the cell pellet, but remove as much supernatant as possible, as this solution contains DMSO.
8. Tap the conical vial to loosen the cell pellet. Re-suspend the pellet very gently by adding 1ml of warm NutriStem® V9 XF medium. Make sure not to break up cell clumps any more than necessary, two gentle pipetting motions are usually sufficient.
9. Slowly add hPSC clumps suspension to the prepared well in the 6-well plate.

10. Place the plate in a 37°C incubator and carefully slide the plate back and forth and side to side to evenly distribute clumps throughout the well. Do not swirl the plate in a circular motion to avoid concentrating the colonies in the center.
11. Allow the cells to attach at 37°C and 5% CO_2 for 2 days.
12. After 48 hours, change the medium daily with 3ml/well culture medium until the colonies are large enough to passage.

Notes:

- Do not move the plate during the first 48 hours post-split (this may increase differentiation of hPSC).
- It is possible to perform a higher volume feed (5-6ml) for the weekend and skip 2 days without changing the medium.

Adaptation to NutriStem® V9 XF medium

Transfer hPSC from a Matrigel-based culture system to Vitronectin, as well as shifting from another hPSC medium to NutriStem® V9 XF, is simple and straightforward.
hPSC may be passaged directly to NutriStem® V9 XF medium on Vitronectin ACF matrix using the enzyme-free passage. Other dissociation methods such as collagenase or dispase are not recommended. Normally, the cells will adapt to the new medium within 2-3 passages.

Critical point to consider before beginning cell adaptation

- Begin cell adaptation with high quality cells; near confluent culture with low background differentiation.
- Timing of passage is critical – culture should be near confluency at the time of passage.
- Seeding density at passage is critical – low split ratio for the first 3 passages of adaptation. A higher proliferation rate will be observed when the cells are fully adapted.
- Morphology change is expected – the edges of colonies may be less compact than the centers when adapting to NutriStem® V9 XF medium.
- Some differentiation may occur and should be removed manually prior to passaging.

Adaptation protocol

1. Prepare a new coated plate according to the coating procedure described above.
2. Pre-warm the required volume of NutriStem® V9 XF needed for this procedure.
3. Detach colonies using 0.5mM EDTA solution in accordance with the passage protocol.
4. Plate the cell aggregates at the desired density onto Vitronectin ACF pre-coated wells with 3ml pre-equilibrated complete NutriStem® V9 XF medium. A 1:3-1:6 split ratio is suggested for the first 3 passages. Adjust split ratio according to culture confluence in each passage.
5. Place the plate in a 37°C CO_2 incubator. Move the plate several times back and forth, and side to side to distribute the aggregates evenly in the well.
6. After 48 hours, change the medium daily with 3ml/well complete NutriStem® V9 XF medium until the colonies are large enough to passage.

Note:
Some differentiation may occur during adaptation and should be removed manually.

Auxiliary Products

Product	Product Cat#
Vitronectin ACF	05-752-002
DPBS, no calcium, no magnesium	02-023-1
DMEM/F-12	01-170-1
CryoStem™ Freezing Medium	05-710-01
EDTA 0.5M Solution	01-862-1

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