Corning® NutriStem® hPSC XF Medium

Corning Cat. Nos. 40-05-100-1A (500 mL) and 40-05-100-1B (100 mL)

Instructions for Use

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Contents

1.0 Introduction

Corning® NutriStem® hPSC XF medium is a defined, xeno-free, serum-free medium designed to support the growth and expansion of human pluripotent stem cells (hPSCs) including human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) in a feeder-free environment. NutriStem hPSC XF Medium offers the ability to culture hPSCs without the need for high levels of bFGF and other stimulatory growth factors or cytokines. The low-protein formulation contains only the most essential components required for maintenance of hPSCs, providing a simplified medium to maintain the cells' full differentiation potential.

The defined, xeno-free formulation of NutriStem hPSC XF medium provides consistent media performance, as well as increased reproducibility for long-term hPSC culture.

2.0 Corning NutriStem hPSC XF Medium Thawing and Handling

Complete and ready to use.

2.1 Medium should be stored in a freezer at -20°C.

2.2 Thaw at room temperature or overnight at 2°C to 8°C. Thawed medium is stable for 2 weeks when stored at 2°C to 8°C. Protect the medium from light.

2.3 Dispense into aliquots to avoid repeated freezing and thawing; freezing/thawing more than 2 times is not recommended.

2.4 NutriStem hPSC XF medium must be pre-warmed to room temperature or 37°C before use. To ensure the stability of the medium, pre-warm only the required volume.

2.5 Different hPSC lines may require protocol optimization for best results.

3.0 Using Corning NutriStem hPSC XF Medium with Corning rLaminin-521 (Human)

3.1 Coating Procedure for Corning rLaminin-521 (Human)

All procedures should be performed under sterile conditions using aseptic techniques.

3.1.1 Thaw rLaminin-521 at 2°C to 8°C before use.

3.1.2 Dilute the thawed rLaminin-521 using 1X Dulbecco's Phosphate-Buffered Saline (DPBS) (with Ca/Mg) to a final concentration of 10 µg/mL Laminin Coating Solution (LCS).

NOTE: The optimal coating concentration is cell-dependent and can be optimized empirically. A concentration of 10 µg/mL supported all hPSC lines tested.

3.1.3 Apply LCS to tissue culture-treated vessels following volumes recommended in Table 1.

Table 1. Recommended Coating Volumes

Vessel	Volume of Laminin Coating Solution (LCS)
6-well plate	1 mL/well
12-well plate	0.4 mL/well
24-well plate	0.2 mL/well
T-75 flask	8 mL
T-175 flask	18 mL

3.1.4 Seal the plates with Parafilm® and incubate the vessel at 2°C to 8°C overnight.

NOTE: Proper sealing is required to prevent evaporation and contamination. Prevent drying out during storage. The rLaminin-521 matrix will be inactivated if allowed to dry. Coated plates can be kept in LCS at 2°C to 8°C for up to 3 weeks if not used immediately.

3.1.5 Prior to cell seeding, aspirate the LCS using a pipet without disturbing the coated surface.

3.1.6 Add NutriStem medium to the coated vessels, and keep in a humidified incubator (37°C, 5% CO_2) until cells are ready to be seeded.

3.2 Single-cell Passaging using Corning® NutriStem® hPSC XF Medium with Corning rLaminin-521 (Human)

3.2.1 Pre-warm all solutions (e.g., NutriStem medium, passaging reagent) as recommended by the manufacturers.

3.2.2 Cells are ready to be passaged when confluency is ≥80% or by day 8, whichever is earlier.

NOTE: Split times may vary for different hPSC lines.

3.2.3 Aspirate the old medium from wells, and wash the cells gently once with 1X DPBS (without Ca/Mg).

3.2.4 Add passaging reagent of choice (e.g., Accutase® or TrypLE™) or 1 mM EDTA diluted in 1X DPBS (without Ca/Mg), and incubate in a humidified incubator (37°C, 5% CO_2) for 3 to 5 minutes (6 to 8 minutes with EDTA) to detach the cells from the surface.

NOTE: Dissociation time may need to be optimized for different hPSC lines.

3.2.5 Pipet up and down 6 to 10 times (as required) to achieve a single-cell suspension. Add the same volume of enzyme inhibitor or fresh medium.

3.2.6 Collect the cell suspension in a conical tube and centrifuge at 100 × g for 4 minutes. Carefully discard/aspirate the supernatant.

3.2.7 Resuspend the cell pellet in 1 mL of fresh medium.

NOTE: When using rLaminin-521 coated vessels for culture, treatment with apoptosis inhibitors such as Rho-kinase (ROCK) or blebbistatin is NOT needed.

3.2.8 Count the cells and seed at a density of 50,000 cells/cm^2 on rLaminin-521 coated vessels.

NOTE: Optimization of seeding density may be required depending on the hPSC line being cultured.

3.2.9 Swing the vessel side to side to distribute cells evenly and then place in a humidified incubator (37°C, 5% CO_2).

3.2.10 Add only a few drops of fresh medium 24 hours after passaging and perform a complete medium change after 48 hours. Feed cells daily until next passage.

4.0 Using Corning NutriStem hPSC XF Medium with Corning PureCoat™ rLaminin-521 Cultureware

Procedures listed below are for hPSC culture on 6-well plates; adjust reagent volumes based on the vessel size.

4.1 Preparation of Corning PureCoat rLaminin-521 Cultureware for Culture

Cultureware is shipped at room temperature but should be stored at 2°C to 8°C until ready for use.

4.1.1 On the day of culture, open the bag in a biosafety hood.

NOTE: PureCoat rLaminin-521 cultureware DOES NOT require any washing before use.

4.1.2 Add 1 mL culture medium/well (or 0.1 mL/cm^2 for other vessel formats) and equilibrate for 30 minutes to 1 hour in a humidified incubator (37°C, 5% CO_2) prior to cell seeding.

⚠ **CRITICAL STEP:** Adding culture media ensures the cell culture surface is pre-wetted before the cells are added to the vessel.

4.2 Single-cell Passaging using Corning NutriStem hPSC XF Medium with Corning PureCoat rLaminin-521 Cultureware

Procedures listed below are for culture in 6-well plate format. Adjust reagent volumes based on the vessel size.

4.2.1 Pre-warm all solutions (e.g., culture medium, dissociation reagent) to room temperature or 37°C as recommended. PureCoat rLaminin-521 cultureware should be pre-equilibrated as described in Section 4.1.3.

4.2.2 Cells are ready to be passaged when they are ~80% confluent or by day 8, whichever is earlier.

4.2.3 Aspirate old medium from the wells and wash the cells gently once with 1X DPBS (without Ca/Mg).

4.2.4 Add passaging reagent of choice (e.g., Accutase® 1 mL/well) and incubate in a humidified incubator (37°C, 5% CO_2) for 3 to 4 minutes to detach cells from the surface.

NOTE: Dissociation times may vary for different hPSC lines and dissociation reagents.

4.2.5 Pipet up and down 6 to 10 times (as needed) to achieve a single-cell suspension. Add fresh medium (3 to 4 mL) to the single-cell suspension to dilute the passaging reagent.

4.2.6 Collect the cell suspension in a conical tube and centrifuge at 100 x g for 4 minutes. Carefully discard/aspirate the supernatant.

4.2.7 Resuspend the cell pellet in 1 mL fresh medium.

NOTE: When using rLaminin-521 cultureware, treatment with apoptosis inhibitors, such as Rho-kinase (ROCK) inhibitor or blebbistatin, is NOT needed.

4.2.8 Count the cells and seed at a density of 50,000 cells/cm^2 on the previously equilibrated Corning® PureCoat™ rLaminin-521 cultureware.

NOTE: Optimization of cell seeding density may be required depending on the culture medium, passaging reagent, and the hPSC line.

4.2.9 Immediately after adding the cell suspension, swing the vessel gently from side to side to distribute the cells evenly, and then place the plate in a humidified incubator (37°C, 5% CO_2).

CRITICAL STEP: Cells will attach very quickly to the PureCoat rLaminin521 surface (<30 sec.). It is critical to make sure cells are mixed and distributed evenly when seeding.

- For 6-well plates, add the cell suspension drop-wise to each well to evenly distribute the cells and shake the plate immediately.
- For flasks, keep the flask vertical when adding the cell suspension. Lay it flat and immediately mix by gently rocking the flask side to side and front to back to distribute the cells evenly.
- Proper mixing should be done prior to moving the vessel into the incubator.

4.2.10 Perform a complete medium change 48 hours after passaging. Feed cells daily until next passage.

NOTE: If the cells are seeded/passaged on Friday, no media change is required over the weekend (Saturday and Sunday).

4.3 Human PSC Morphology using Corning NutriStem® hPSC XF Medium with Corning PureCoat rLaminin-521 Cultureware

The cells attach and form small islands or colony-like structures and initially appear to have an enlarged appearance (not typical of hPSCs). After day 4, the colonies start to merge and form a monolayer (Figure 1). At this point, the cells possess typical undifferentiated hPSC morphology with prominent nuclei and high nucleus-to-cytoplasm ratio.

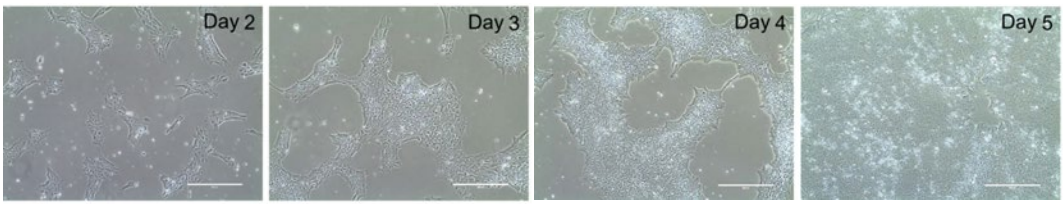

Figure 1. Gibco Human Episomal iPSC Line on Corning PureCoat rLaminin-521 cultureware in Corning NutriStem hPSC XF culture medium. Images at 10X magnification (scale bar = 400 µm).

5.0 Using Corning® NutriStem® hPSC XF Medium with Corning Matrigel® hESC-qualified Matrix

Corning Matrigel hESC-qualified matrix should be aliquoted, and stored as recommended in the product Guidelines for Use.

5.1 Coating Procedure for Corning Matrigel hESC-qualified Matrix

All procedures should be performed under sterile conditions using aseptic techniques. Matrigel hESC-qualified matrix, coating medium, tubes, pipet, tips, etc. must be kept ice-cold during the entire procedure.

5.1.1 Thaw an aliquot of Matrigel hESC-qualified matrix on ice or at 2°C to 8°C.

5.1.2 Dilute the thawed Matrigel hESC-qualified matrix using DMEM/F-12. The dilution factor for Matrigel hESC-qualified matrix is lot number specific and available on the Certificate of Analysis.

5.1.3 Add 1 mL of diluted Matrigel hESC-qualified matrix per well of a 6-well plate.

5.1.4 Swirl the plate to distribute the Matrigel hESC-qualified matrix solution evenly across the surface.

5.1.5 Incubate the vessels at room temperature for at least 1 hour before use.

NOTE: Coated plates can be wrapped in Parafilm® and stored at 2°C to 8°C for up to 1 week, if not used immediately. Proper sealing is required to prevent contamination and evaporation/drying out during storage.

5.1.6 Prior to cell seeding, bring the coated vessel to room temperature, and aspirate the coating solution using a pipet without disturbing the coated surface.

5.2 Clump Passaging using Corning NutriStem hPSC XF Medium with Corning Matrigel hESC-qualified Matrix

5.2.1 Pre-warm all solutions (e.g., NutriStem medium, passaging reagent) as recommended by the manufacturers.

5.2.2 Cells are ready to be passaged when confluence is ~80%.

NOTE: Split times may vary for different hPSC lines. Typically hPSC colonies are ready to passage when the colonies are large, beginning to merge, and have centers that are dense and phase-bright compared to their edges. Depending on the size and density of seeded aggregates, cultures are usually passaged 3 to 5 days after seeding in NutriStem medium.

5.2.3 Aspirate old medium from the wells and wash the cells gently once with 2 mL of DPBS (without Ca/Mg).

5.2.4 Add 1 mL of dissociation reagent/well (e.g., cell dissociation buffer) and incubate at room temperature until separation of the cells within the colonies is observed under the microscope (3 to 5 minutes).

NOTE: Dissociation time may need to be optimized for different hPSC lines.

5.2.5 Carefully aspirate cell dissociation buffer from the wells and dislodge the cells from the plate as small cell clumps by forcefully adding fresh medium to the well using a P1000 PIPETMAN®.

NOTE: Avoid excessive/forceful trituration to preserve the clumps and prevent single cell formation.

5.2.6 Add the cells to a tube containing 2 mL fresh medium, and rinse the well with 3 to 5 times more medium based on the desired split ratio.

5.2.7 Seed aliquots of cell clumps onto a Matrigel matrix-coated plate at the desired split ratio in the NutriStem hPSC XF medium (3 mL/well). Evenly distribute the cell clumps within the wells by moving the plate side to side and front to back and incubate at 37°C, 5% CO_2 in a humidified incubator.

NOTE: Optimization of seeding density may be required depending on the hPSC line being cultured.

5.2.8 Change medium on day 2 by removing the old medium and adding 3 mL fresh medium in each well. Perform medium change daily until next passage.

6.0 Ordering Information

Cat. No.	Description	Size	Qty/Pk
40-05-100-1A	Corning® NutriStem® hPSC XF medium, [+] HSA	500 mL	1
40-05-100-1B	Corning NutriStem hPSC XF medium, [+] HSA	100 mL	1

Related Media Products

Buffers and Reagents			
10-090-CV	DMEM (Dulbecco's Modification of Eagle's Medium)/Ham's F-12 50/50 Mix, [+] L-glutamine	500 mL	6
21-030-CV	DPBS (Dulbecco's Phosphate Buffered Saline), 1x, [+] calcium, magnesium	500 mL	6
21-031-CV	DPBS (Dulbecco's Phosphate Buffered Saline), 1x, [-] calcium, magnesium	500 mL	6
25-058-CI	Accutase® cell detachment solution	100 mL	1
Surfaces			
354220	Corning rLaminin-521 (Human), 20 µg vial	20 µg	1
354221	Corning rLaminin-521 (Human), 100 µg vial	100 µg	1
354277	Corning Matrigel® hESC-qualified matrix	5 mL	1
rLaminin Coated Vessels			
356290	Corning PureCoat™ rLaminin 6-well plate	5	5
Vessels			
3516	Corning 6-well clear TC-treated multiple well plate, individually wrapped, sterile	1	50
3513	Corning 12-well clear TC-treated multiple well plate, individually wrapped, sterile	1	50
3524	Corning 24-well clear TC-treated multiple well plate, individually wrapped, sterile	1	50
430641	Corning 175 cm² cell culture flask, canted neck, vented cap	5	100
431080	Corning 75 cm² U-shaped cell culture flask, canted neck, vented cap	5	50

7.0 Certificate of Conformance

Quality certificates can be obtained at **www.corning.com/lifesciences**. Catalog and lot number required.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

Warranty/Disclaimer: NutriStem is for research use or further manufacturing use only. Unless otherwise specified, all other products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

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