

Corning® NutriStem® hPSC XF Medium

Cat. Nos.: 40-05-100-1A (500 mL), 40-05-100-1B (100 mL)

Frequently Asked Questions

The CORNING logo is located in the top right corner of the page. It consists of the word "CORNING" in a white, uppercase, sans-serif font, centered within a solid orange square.

1. What is Corning NutriStem hPSC XF medium?

NutriStem hPSC XF medium is a serum-free and xeno-free medium that supports long-term culture of human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). The cells remain undifferentiated, maintain pluripotency, and a normal karyotype.

2. How should NutriStem hPSC XF medium be prepared?

Medium is shipped frozen as complete medium and is ready to use after thaw.

3. How should NutriStem hPSC XF medium be stored prior to use?

The NutriStem hPSC XF medium is stored in a freezer (-20°C).

4. How should NutriStem hPSC XF medium be thawed?

The medium should be thawed at 2°C to 8°C or at room temperature.

5. Can NutriStem hPSC XF medium be aliquoted and refrozen?

Post-thaw, medium can be aliquoted to appropriate work volumes which should be re-frozen. Expiry date is as indicated on the original bottle.

6. How many freeze-thaw cycles can be performed to NutriStem hPSC XF medium?

The medium can be thawed twice – the first thaw when making aliquots from the original bottle and the second thaw when working with the medium.

7. What is the shelf-life after thawing the NutriStem hPSC XF medium?

Thawed medium can be stored for up to two weeks at 2°C to 8°C, protected from light.

8. How should the NutriStem hPSC XF medium be pre-warmed before use?

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.

9. Do I need to add supplements to NutriStem hPSC XF medium before use?

NutriStem hPSC XF medium is a complete medium, no supplements are needed.

10. Do I need to add bactericides and/or fungicides to the NutriStem hPSC XF medium?

The use of antimicrobial agents is not required if workflow and culture environments are kept sterile.

11. Can ROCK inhibitors be used with NutriStem hPSC XF medium?

For single cell culture on Corning rLaminin-521 (Human) and Corning PureCoat™ rLaminin-521 cultureware, addition of ROCK inhibitor is NOT required. If culturing single cells on Corning Matrigel® hESC-qualified matrix, ROCK inhibitor can be added.

12. Is NutriStem hPSC XF medium manufactured in a cGMP environment?

NutriStem hPSC XF medium is manufactured in a cGMP facility.

13. What quality control testing is done on NutriStem hPSC XF medium?

Each batch is tested for hPSC culture and expression of undifferentiated hPSC markers. Other testing includes pH, osmolality, mycoplasma detection, endotoxin, and sterility.

14. Can hiPSCs be generated using Corning® NutriStem® hPSC XF medium?

Using different somatic cell types and reprogramming methods hiPSCs have been generated and transferred into NutriStem hPSC XF medium.

- ▶ Warren, L., et al. (2010). Cell Stem Cell 7:618-630
- ▶ Rajasingh S., et al. (2015). PLoS ONE 10(8):e0134093. doi:10.1371/journal.pone.0134093

15. Can hPSCs previously cultured in other media and on other substrates be cultured in NutriStem hPSC XF medium?

Yes.

16. Can hPSCs cryopreserved from a different culture condition be thawed and subsequently cultured in NutriStem hPSC XF medium?

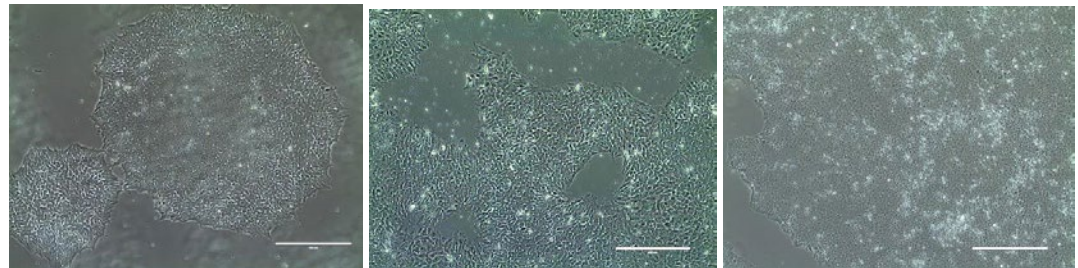
Yes.

17. What surfaces are recommended for culture of hPSCs with NutriStem hPSC XF medium?

Corning Matrigel® hESC-qualified matrix supports clump passaging of hPSCs in NutriStem hPSC XF medium without ROCK inhibitor or single cell passaging with ROCK inhibitors. Corning rLaminin-521 (Human) and Corning PureCoat™ rLaminin-521 cultureware can be used for single cell culture of hPSCs without ROCK inhibitor.

18. What will cells look like growing in NutriStem hPSC XF medium?

Phase contrast Images of hiPSCs on different surfaces in NutriStem hPSC XF medium are shown below.



Corning Matrigel hESC-qualified matrix

Corning rLaminin-521 (Human)

Corning PureCoat rLaminin-521

19. How often is medium change required?

- ▶ **Corning rLaminin-521 (Human):** 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.
- ▶ **Corning PureCoat rLaminin-521 cultureware and Corning Matrigel hESC-qualified matrix:** Cells need to be fed after 48 hours of passaging. If cells are passaged on Friday, weekend media change can be skipped. Afterwards, medium should be changed daily until cells are ready for passage.

20. When should hPSCs be sub-cultured?

- ▶ **Corning rLaminin-521 (Human) and Corning PureCoat rLaminin-521 cultureware:** Cells should be passaged when they are at ~80% confluence which typically require passaging every 3 to 5 days.
- ▶ **Corning Matrigel hESC-qualified matrix:** 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 hPSC XF medium.

Passaging time may vary depending on the hPSC line and passaging method and needs to be optimized by the end-user.

21. What reagent should be used for dissociation of pluripotent cells?

Accutase® is recommended when passaging as single cells on Corning® PureCoat™ Laminin-521 cultureware. For single cell passaging on Corning rLaminin-521 (Human) dissociation reagents such as TrypLE™ or Accutase can be used. Clump passaging on Corning Matrigel® hESC-qualified matrix can be performed using cell dissociation reagents such as EDTA or Dispase.

22. What is the recommended sub-culturing ratio when using Corning NutriStem® hPSC XF medium?

Split ratio for Corning Matrigel hESC-qualified matrix and seeding density for Corning rLaminin-521 (Human) and Corning PureCoat rLaminin-521 cultureware should be optimized based on hPSC line. We recommend a starting seeding density of 50,000 cells/cm² for hPSC culture on Corning rLaminin-521 (Human) and Corning PureCoat rLaminin-521 cultureware.

23. What hPSC lines have been successfully cultured with NutriStem hPSC XF medium under feeder-free conditions?

Several hESC lines (e.g., HS980, HS181, H9, HES2, KhES-1, KhES-3) and hiPSC lines (e.g., BC-1, C3, Gibco® Human Episomal iPSC line) have been cultured in NutriStem hPSC XF medium.

24. Do cells need adaptation when they are transferred to NutriStem hPSC XF medium?

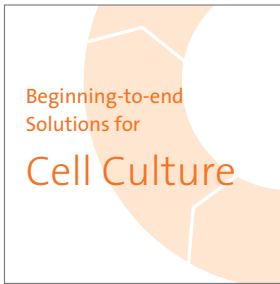
Most hPSC lines will not require adaptation, simply seed cells directly into NutriStem hPSC XF medium. Some hPSC lines may require gradual adaptation for first few passages.

25. What should be done if sediments appear in the NutriStem hPSC XF medium after thawing?

NutriStem hPSC XF medium does not contain ingredients that cause sedimentation and therefore, the medium should not be used if sediments are observed after thawing.

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

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