Corning® PureCoat™ rLaminin-521 Cultureware

Frequently Asked Questions

CORNING

1. **How is Corning® PureCoat™ rLaminin-521 cultureware made?**

   Corning PureCoat rLaminin-521 cultureware surfaces are made using a proprietary Corning coating technology. Recombinant Laminin-521 is passively-bound to the surface.

2. **What is the "animal-free" claim for Corning PureCoat rLaminin-521 cultureware?**

   The product does not contain materials of animal origin.

3. **What are the storage requirements for Corning PureCoat rLaminin-521 cultureware?**

   PureCoat rLaminin-521 cultureware is shipped at room temperature, but it should be stored at 2°C to 8°C.

4. **How soon after I open the package do I have to work with the vessels?**

   The package should be opened just prior to use.

5. **What is the shelf-life of this product?**

   Shelf-life (for unopened product) is 20 months at 2°C to 8°C.

6. **What is the shelf-life of the product after I open the package?**

   Shelf-life is based on unopened product. The product should be used immediately after opening.

7. **Does PureCoat rLaminin-521 cultureware need to be washed before use?**

   PureCoat rLaminin-521 cultureware is ready-to-use. No washing is required before use. Keep the product sealed in original packaging until it is ready to use.

8. **Does PureCoat rLaminin-521 cultureware need to be pre-equilibrated before use?**

   CRITICAL STEP

   PureCoat rLaminin-521 cultureware needs a pre-equilibration or pre-wetting step just prior to use. Add appropriate volume of desired cell culture medium to the vessels (as described in the *Guidelines for Use*) and pre-equilibrate the vessels for 30 minutes to 1 hour in a humidified incubator at 37°C, 5% CO_2 before seeding the cells.

9. **Can I use any unused wells of a PureCoat rLaminin-521 cultureware 6-well plate?**

   Stability and sterility of the unused wells might be compromised in the incubator or during handling. We recommend using a new plate for the next experiment.

10. **What cell types have been cultured on Corning PureCoat rLaminin-521 cultureware?**

    Human pluripotent stem cells (hPSCs) and human neural stem cells (hNSCs) have been cultured on PureCoat rLaminin-521 cultureware. The cultureware has also been used for the derivation/generation of human-induced pluripotent stem cells (hiPSCs), culture of retinal pigment epithelium (RPE) cells, and differentiation of hNSCs.

11. **What commercially available cell culture media are recommended for hPSC culture?**

    Culture media such as mTeSR™1 (STEMCELL Technologies), NutriStem® XF/FF culture medium (Stemgent), StemMACS™ iPS-Brew XF (Miltenyi Biotec), XVIVO™ 10 (with supplements; Lonza) and E8 medium (Thermo Fisher Scientific or STEMCELL Technologies) have been used for hPSC culture on PureCoat rLaminin-521 cultureware.

12. **What commercially available cell culture media have been used for hNSC culture?**

    Human NSCs have been cultured with StemPro® NSC SFM (Thermo Fisher Scientific).

13. How long does it take for cells to attach to Corning® PureCoat™ rLaminin-521 cultureware?

CRITICAL STEP

Cells begin to attach within 30 seconds on PureCoat rLaminin-521 cultureware. It is imperative to ensure the cell suspension is mixed well and the cells are distributed evenly on the cell culture surface, immediately after addition to the vessel.

For the 6-well plate, add the cell suspension drop-wise to each well to evenly distribute the cells and immediately shake the plate gently.

For flasks, keep the flask vertical when adding the cell suspension. Lay it flat and immediately shake it from side-to-side and front-to-back to distribute the cells evenly. This step should be done before moving the flask into the incubator.

14. When hPSCs are cultured on PureCoat rLaminin-521 cultureware will they look morphologically similar to other feeder-free substrates (e.g., Corning Matrigel® matrix)?

There are slight differences in morphology of hPSCs right after the cells attach to the PureCoat rLaminin-521 cultureware surface. Until day 3 or 4, the cells will look slightly enlarged and less compacted. However, by day 4 or 5, they exhibit typical hPSC morphology with prominent nuclei, small cell size, and high nucleus to cytoplasm ratio.

15. Can hPSCs be thawed on PureCoat rLaminin-521 cultureware?

Cells can be directly thawed onto the PureCoat rLaminin-521 cultureware as either clumps or single cell suspension. In some instances, an adaptation step might be required if the cells were originally frozen from a different medium-surface culture system. The cells might need to be thawed onto their original surface and then transferred to PureCoat rLaminin-521 cultureware.

16. What passaging methods have been used to culture hPSCs on Corning PureCoat rLaminin-521 cultureware?

We recommend single cell passaging of hPSCs on PureCoat rLaminin-521 cultureware. However, clump passaging has also been successfully demonstrated.

17. What is the recommended cell seeding density for single cell passaging of hPSCs on Corning PureCoat rLaminin-521 cultureware?

We recommend a cell seeding density of 50,000 cells/cm^2 for hPSC culture as a single cell suspension. However, seeding density can be optimized by the end user based on hPSC line and culture media.

18. What cell dissociation reagents are recommended for single cell passaging of hPSCs on Corning PureCoat rLaminin-521 cultureware?

We recommend the use of Accutase™ for single cell passaging on PureCoat rLaminin-521 cultureware.

19. Is the addition of small molecules (such as ROCK inhibitor) required during single cell passaging of hPSCs on Corning PureCoat rLaminin-521 cultureware?

No, the addition of small molecules (such as ROCK inhibitors) are not required during single cell passaging on PureCoat rLaminin-521 cultureware.

20. What is the optimal passaging time for single cell passaging of hPSCs?

Passaging times should be optimized based on the cell culture conditions. Typically, cells are ready to be passaged between days 4 to 8 or when they reach a confluence of ~80%.

21. What techniques of clump passaging of hPSCs have been tested on Corning PureCoat rLaminin-521 cultureware?

We recommend the use of EDTA-based cell dissociation reagents for clump passaging on PureCoat rLaminin-521 cultureware. Alternatively, solely mechanical passaging with a sterile cell scraper or pipet tip can be performed. Use of Dispase is not recommended.

22. Do hPSCs (previously cultured on a different substrate) undergo an adaptation period after they are transferred onto Corning® PureCoat™ rLaminin-521 cultureware?

Cells can be directly transferred and cultured on PureCoat rLaminin-521 cultureware. Human PSCs might undergo an adaptation period during the initial three passages in PureCoat rLaminin-521 cultureware.

23. Is this product manufactured in a cGMP environment?

PureCoat rLaminin-521 cultureware are manufactured in a facility compliant with the current version of the EN ISO 9001 Standard, the EN ISO 13485 Standard, and the FDA CFR 21 Part 820, current Good Manufacturing Practices (cGMP).

24. What is the quality control for this product?

Nonpyrogenic: Products tested and met the criteria established in the current version of United States Pharmacopeia (USP) Chapter <85>,"Bacterial Endotoxins Test." The acceptance level for product is ≤0.5 EU/mL or ≤20 EU/device.

Functionality: Corning PureCoat rLaminin-521 cultureware have been tested for the ability to support human induced pluripotent stem cell attachment, expansion, and single cell passaging in serum-free media.

Sterility: Corning PureCoat rLaminn-521 cultureware have been manufactured using aseptic processing per ISO 13408-1, "Aseptic Processing of Healthcare Products" and tested according to USP <71> "Sterility tests." Each step has been validated to ensure that all products meet the industry standard Sterility Assurance Level (SAL) of 10^{-3}.

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

Warranty/Disclaimer: Unless otherwise specified, all 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.

www.corning.com/lifesciences/solutions

At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

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