

1. Product Background

Laminin 521 (LN521) is a recombinant human protein that provides a defined surface for in vitro feeder-free culture of multiple human pluripotent stem cells (PSCs). LN521 has been proven to maintain normal growth characteristics and stemness in multiple PSC lines without simultaneous differentiation, which includes ESC, iPSC, MSC etc. In addition, LN521 has been demonstrated to support PSC growth for >10 passages without any signs of karyotypic abnormalities and to maintain the ability of PSCs to differentiate into all three germ line lineages.

2. Experimental instruments and materials

2.1 Instruments: Biosafety cabinet, cell incubator, low temperature horizontal centrifuge, inverted microscope.

2.2 Materials: Sterile pipette tips; Sterile EP tube and other consumables.

2.3 Reagents: Laminin-521(GMP-LA5H24), ROCK inhibitor(Y-27632), DMEM F12, mTeSR-plus medium, ReLeSR, PBS(Ca^{++} / Mg^{++}) (Cytiva Cat. SH30256.01) , D-PBS (Ca^{++} / Mg^{++})(Cytiva Cat. SH30264.01).

3. Experimental contents and methods

3.1. Laminin plate coating:

3.1.1. Use sterile deionized water or water for injection to reconstitute laminin-521 to 400µg/ml. It is recommended to further dilute to **100µg/ml** with sterile D-PBS(Ca⁺⁺/Mg⁺⁺) prior to use, and then dilute to working concentration of **5µg/ml** with sterile D-PBS(Ca⁺⁺/Mg⁺⁺) . When culture hPSCs for the first time, a higher working concentration is recommended to adapt the new matrix.

NOTE: DPBS with Ca²⁺ and Mg²⁺ should be used since divalent cations are important for the protein structure and function.

*The required working concentration of Laminin 521 is cell and application dependent. We recommend using an initial coating concentration of **0.5 µg/cm²** on the culture surface. The detailed guidance for calculating the adding volumes of Laminin 521 is referred to the Table 1.*

Culture vessel	Coating concentration	Volume (laminin)	Volume (DPBS)	Total volume	mTeSR-plus media
6-well	0.5 $\mu\text{g}/\text{cm}^2$	50 $\mu\text{L}/\text{well}$	950 $\mu\text{L}/\text{well}$	1 mL/well	2 mL
12-well	0.5 $\mu\text{g}/\text{cm}^2$	25 $\mu\text{L}/\text{well}$	475 $\mu\text{L}/\text{well}$	0.5 mL/well	1 mL
24-well	0.5 $\mu\text{g}/\text{cm}^2$	15 $\mu\text{L}/\text{well}$	285 $\mu\text{L}/\text{well}$	0.3 mL/well	0.5 mL
35-mm	0.5 $\mu\text{g}/\text{cm}^2$	50 $\mu\text{L}/\text{well}$	950 $\mu\text{L}/\text{well}$	1 mL/well	2 mL
60-mm	0.5 $\mu\text{g}/\text{cm}^2$	100 $\mu\text{L}/\text{well}$	1900 $\mu\text{L}/\text{well}$	2 mL/well	4 mL
100-mm	0.5 $\mu\text{g}/\text{cm}^2$	300 $\mu\text{L}/\text{well}$	5700 $\mu\text{L}/\text{well}$	6 mL/well	12mL

The above volume is calculated according to the working concentration of 5 $\mu\text{g}/\text{mL}$.

For example, for one well of a 12-well plate, add 25 μL of laminin 521 (2.5 μg) in 475 μL DPBS($\text{Ca}^{++}/\text{Mg}^{++}$) . Then, add 0.5 mL of diluted Laminin 521 solution to the well.

3.1.2. Add indicated volumes of the laminin-DPBS mixture into the each well and gently shake to ensure that matrix is spread across the well.

3.1.3. Transfer the plate into a 4° C refrigerator for incubation overnight or 37° C incubator for at least 2 hours. Overnight is recommended for ideal cell culturing conditions. Do not allow the culture vessel to dry.

3.1.4. Aspirate the Laminin 521 solution and discard when cells are ready to be plated.

4. Cell Culture - iPSC

4.1. Thawing of iPSCs

4.1.1. Remove iPSCs from storage in liquid nitrogen or dry ice and thaw in 37° C water for 10s.

4.1.2. Disinfect frozen tube with 75% alcohol and transfer it onto the benchtop.

4.1.3. Transfer cell solution into a new 15 mL Centrifuge tube with DMEM-F12 of 9 ml.

4.1.4. Centrifuge the 15 mL Centrifuge tube at room temperature at 300g for 5 minutes.

4.1.5. Discard the well-coated laminin solution in 12 well plate.

4.1.6. Discard the cell supernatant and gently resuspend iPSCs with 1 mL mTeSR-plus containing 10 μ M Y-27632, and transfer it to a coated 12 well plate. Shake plate to evenly distribute cells, and stand at room temperature for 10 minutes.

4.1.7. Place the 12-well plate back into 37° C incubator.

4.1.8. ROCK inhibitor-containing medium should be removed after 12-16 h and continue to be cultured in non-inhibitor-containing medium.

4.2. iPSC passaging protocol

4.2.1. Discard the culture supernatant and rinse with 1 mL PBS($\text{Ca}^{++}/\text{Mg}^{++}$) .

NOTE: PBS without Ca^{2+} and Mg^{2+} should be used since divalent cations have a negative effect on some dissociating enzymes.

4.2.2. Discard the PBS and add 0.5 mL ReLeSR. Stand at 37°C for 8-10 minutes or keep under microscopic observation until cells are not bound to the plate.

NOTE: For the passage of induced pluripotent stem cells (iPSCs), TrypLE Select, ReLeSR, and Accutase are also recommended. When dissociating iPSCs and performing pipetting, the digestion process should be carried out at 37°C for approximately 8 to 10 minutes. In the case of iPSC passage in a cell factory without pipetting, it is advisable to either extend the digestion time to 15 - 20 minutes or reduce the coating concentration to around 3 µg/ml.

4.2.3. Add equal volume of DMEM-F12, gently mix the cells and transfer it into a centrifuge tube at room temperature at 300g for 5 minutes.

4.2.4. Prepare a laminin coated 12-well plate before passaging your cells.

4.2.5. Discard the supernatant and resuspend cells with mTeSR-plus medium with 10 µM Y-27632.

4.2.6. Transfer the cells to the prepared laminin-coated 12-well plate. Shake the plate gently to evenly distribute cells, and stand at room temperature for 10-20 minutes.

4.2.7. Place the 12-well plate into a 37° C incubator for incubation.

4.3. Cryopreservation of iPSCs

4.3.1. Prepare ReLeSR for use.

4.3.2. Follow the iPSC passaging protocol for cell isolation. Use a cell counter to ensure cell density before cryopreservation.

4.3.3. Each tube of cells should be frozen at a density of 1×10^6 . Follow the centrifugation, extraction of cell medium steps in the iPSC passaging protocol. Resuspend isolated iPSC pellet in the appropriate volume of cryopreservation solution.

4.3.4. Add 1mL of the resuspended cell cryoprecipitate into a 1.5 mL freezing tube and undergo programmed cooling before transfer into liquid nitrogen for long-term storage.

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