BIOSYSTEMS Acro | Where Proteins and Innovation Advance Biomedicine

Human Laminin 511 Protein, premium grade (Cat. No. LA8-H5283L) Stem Cell Culture Protocol

Scan this QR code for special gifts & more!

1. Product Background

Laminin 511 E8 (LN511-E8) is a recombinant human protein that provides a defined surface for in vitro feeder-free culture of multiple human pluripotent stem cells (PSCs). Being a truncated form of laminin 511, LN511-E8 serves as a functionally minimal form that retain the full capability for binding to integrins. LN511-E8 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, LN511-E8 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. As published by Takamichi Miyazaki et al. , the LN511-E8 variant of laminin 511 shows higher efficiency for supporting the adhesion of dissociated cells than did wild-type laminin 511 which makes a cost-effective choice.

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-511(LA8-H5283L), ROCK inhibitor(Y-27632), DMEM-F12; mTeSR-plus medium; ReleSR, PBS(Ca^{--}/Mg^{--}) (Cytiva Cat. SH30256.01).

3. Experimental contents and methods

3.1. Laminin plate coating:

3.1.1. Slowly thaw Laminin 511 stock solution at 2℃ to 8 ℃.

3.1.2. Gently mix the Laminin 511 stock solution by up-down reversal.

3.1.3 Dilute Laminin 511 stock solution to working concentration of **0.55-1 *μg/cm²*** with sterile PBS(Ca^{--}/Mg^{-}) . When culture hPSCs for the first time, a higher working concentration is recommended to adapt the new matrix.

*Note: The required working concentration of Laminin 511 varies depending on the cell type and specific application. We recommend an initial coating concentration of **0.55 μg/cm²** on the culture surface, which can be further optimized based on the specific cell line and experimental needs. The detailed guidance for calculating the adding volumes of Laminin 511 is referred to the Table 1.*

Culture vessel	Coating concentration	Laminin Amount (µg)	Total Volume (PBS + Laminin)	mTeSR-plus media
6-well	0.55 µg/cm^2	5.3	2 mL/well	2 mL
12-well	0.55 µg/cm^2	2.5	1 mL/well	1 mL
24-well	0.55 µg/cm^2	1.1	0.6 mL/well	0.5 mL
35-mm	0.55 µg/cm^2	4.4	2 mL/well	2 mL
60-mm	0.55 µg/cm^2	11.6	4 mL/well	4 mL
100-mm	0.55 µg/cm^2	30.3	12 mL/well	12mL

For example, for one well of a 6-well plate, add 53µL of laminin 511 (5.3 µg) in 1947µL PBS(Ca^{--}/Mg^{-}) *. Then, add 2 mL of diluted Laminin 511 solution to the well.*

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

3.1.5. Transfer the plate into a 37 ℃ incubator for at least 2 hours. 4 ℃ Overnight is recommended for ideal cell culturing conditions. Do not allow the culture vessel to dry.

3.1.6. Aspirate the Laminin 511 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 μM 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(Ca^{--}/Mg^{--}) .

NOTE: PBS without Ca2+ and Mg2+ 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℃ 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℃ *for approximately 8 to 10 minutes.*

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.

Where PROTEINS and INNOVATION Advance BIOMEDICINE

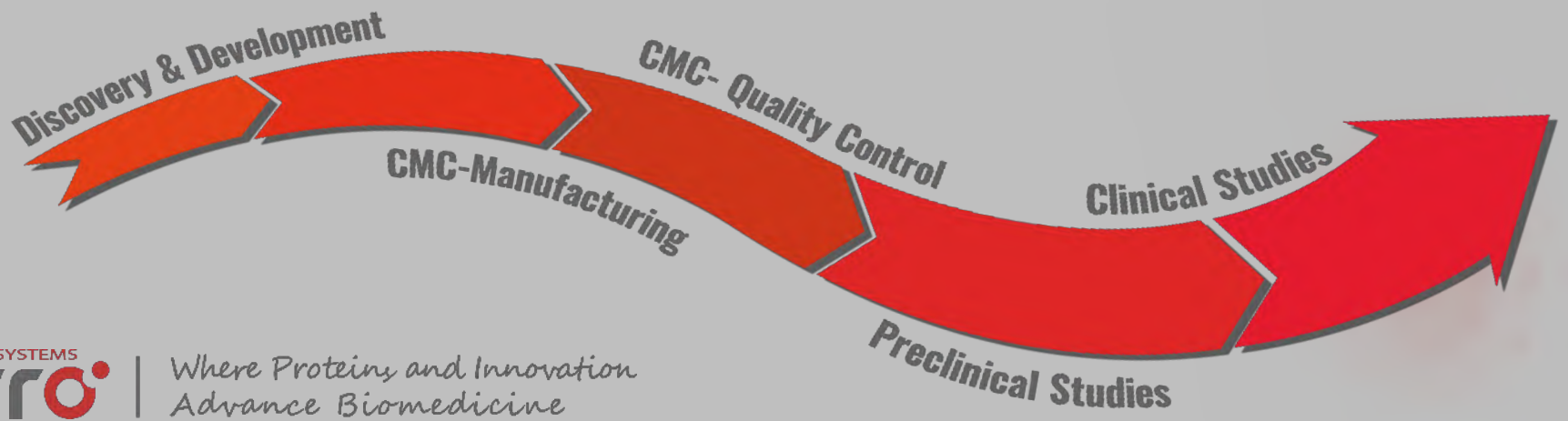
Cell Therapies

Gene Therapies

Vaccine

Antibody-Drug Conjugates

Therapeutic Antibodies



Scan this QR code for special gifts & more!

BIOSYSTEMS Acro | Where Proteins and Innovation Advance Biomedicine