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Human Laminin 511 Protein, premium grade (Cat. No. LA8-H5283) Stem Cell Culture Protocol

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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-H5283), ROCK inhibitor(Y-27632), DMEM-F12; mTeSR-plus medium; Gentle Cell Dissociation Reagent, 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-511 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 **5-10µg/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 Ca^{2+} and Mg^{2+} should be used since divalent cations are important for the protein structure and function. The required working concentration of Laminin 511 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 511 is referred to the Table 1.*

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

The above volume is calculated according to the working concentration of 5 μg/ml.

For example, for one well of a 12-well plate, add 25μL of laminin 511 (2.5 μg) in 475μL DPBS(Ca^{++}/Mg^{++}) . Then, add 0.5 mL of diluted Laminin 511 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 37° C incubator for incubation overnight. The minimal amount of time for use is 2 hours, but overnight is recommended for ideal cell culturing conditions. Do not allow the culture vessel to dry.

3.1.4. 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 for a final cell density of 1×10^5 cells per well, and stand at room temperature for 10 minutes.

Note: Seed the plate according to the cells in the table, determine the number of cells according to the growth rate of your cultured cells, and ensure passage in 4-5 days.

Culture vessel	6 well	12 well	24 well	96 well
Cell numbers	$2.5\sim3.5\times10^5$	$6\sim8\times10^4$	$3\sim4\times10^4$	$0.5\sim1\times10^4$

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 Gentle Cell Dissociation Reagent. Stand at room temperature for 6-8 minutes or keep under microscopic observation until cells are not bound to the plate.

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.

Note: iPSCs will rapidly differentiate and die when they grow into a single layer. In order to maintain growth and pluripotency, make sure to passage your cells before 100% cell confluency.

4.3. Cryopreservation of iPSCs

4.3.1. Prepare Gentle Cell Dissociation Reagent 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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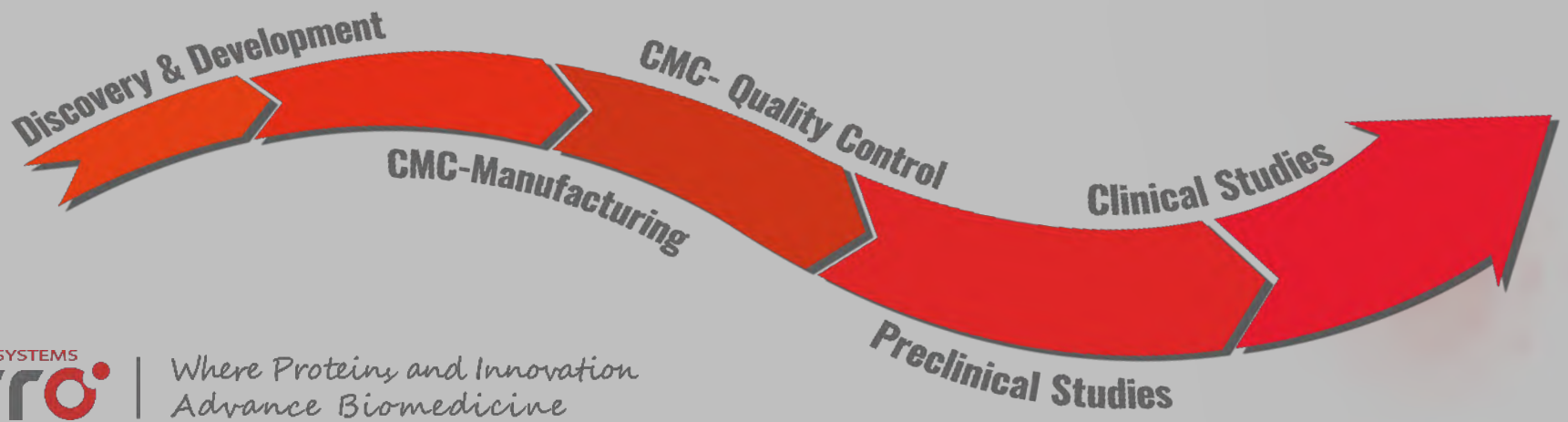

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