Corning® rLaminin-521 (Human)

Catalog Nos. 354220 & 354221

Guidelines for Use

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
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For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Corning® rLaminin-521 (Human)

Coating Procedure

1. All procedures should be done under sterile conditions using aseptic techniques.

2. Thaw rLaminin-521 at 4^0C before use.

3. Dilute the thawed rLaminin-521 using 1x DPBS (with Ca/Mg) to a final concentration of 10 µg/ml Laminin Coating Solution (LCS).

The optimal coating concentration is cell-dependent and can be optimized empirically. A concentration of 10 µg/ml supported all human pluripotent stem cell lines (hPSC) tested.

4. Apply LCS to a tissue culture-treated vessel following recommendations from Table 1.

Table 1. Recommended coating volumes

Vessel	Laminin Coating Solution (LCS)
6 well plate	1 ml/well
12 well plate	0.4 ml/well
24 well plate	0.2 ml/well
T-75 flask	8 ml
T-175 flask	18 ml

5. Seal the plates with parafilm and store at 4^0C overnight.

Proper sealing is required to prevent evaporation and contamination. Prevent drying-out during the storage. The rLaminin-521 matrix will be inactivated if let dry. Coated plates can be kept in LCS at 4^0C for up to 3 weeks if not used directly.

6. Aspirate the LCS using a pipette without disturbing the coated surface.

7. Add culture medium to the coated vessels and keep in a humidified incubator at 37^0C, 5% CO_2 during passaging procedure until cells are ready to be seeded.

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Single-cell passage of hPSC on rLaminin-521

1. When using rLaminin-521, treatment with apoptosis inhibitors such as Rho-kinase (ROCK) or blebbistatin is NOT needed.

2. All procedures should be done under sterile conditions using aseptic techniques.

3. Before start, all solutions (e.g., culture medium, 1x DPBS) should be pre-warmed at 37^0C.

   Cells are ready to be passaged when ≥ 80% confluent or by day 8 whichever is earlier. Aspirate old medium from wells and wash the cells gently once with 1x DPBS (without Ca/Mg).
   Split time may vary for different hPSC lines.

4. Add enzyme of choice (i.e., TrypLE™) or 1 mM EDTA diluted in 1x DPBS (without Ca/Mg) and incubate in a humidified incubator at 37^0C, 5% CO_2 for 3-5 minutes (6-8 minutes with EDTA) to detach cells from the surface.
   Dissociation time may vary for different hPSC lines.

5. Add same volume of enzyme inhibitor or fresh medium and pipette up and down 6 -10 times (as appropriate) to achieve single-cell suspension.

6. Collect the cell suspension in a conical tube containing 1 ml of fresh medium, centrifuge at 800 rpm for 4 minutes. Carefully discard the supernatant.

7. Re-suspend the cell pellet in 1 ml of fresh medium.

8. Count cell number and seed cells at a density of 50,000 cells/cm^2 on rLaminin-521 coated vessels. *Optimization of seeding density may be required depending on the culture medium and hPSC line.*

9. Swing the vessel side-to-side to distribute cells evenly, and then place in a humidified incubator at 37^0C, 5% CO_2.

10. Feed cells daily until next passaging. Add only a few drops of fresh medium after 24 hours and perform a complete medium change 48 hours after passaging.

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From feeders to rLaminin- 521 (single-cell passage)

1. Separate hPSC colonies from feeders and cut into small pieces.

2. Aspirate the free-floating cell aggregates carefully with a pipette.

3. Collect in a conical tube, add enzyme, and incubate in a humidified incubator at 37^0C, 5% CO_2 a bit longer than usual (e.g., trypsin 4-7 minutes).

4. Pipette cell-aggregates up and down to achieve a homogenous cell suspension, centrifuge for 4 minutes at 800 rpm, and discard the supernatant. Re-suspend the cell pellet in fresh culture medium.

5. Seed cells at a density of 50,000 cells/cm^2 on rLaminin-521 coated vessels.

This transfer can also be performed using colony passage for the first transition followed by singe-cell passage.

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