Recombinant Human Laminin-521 (rhLaminin-521)

Description

rhLaminin-521 is a recombinant human protein that provides a defined surface for feeder-free culture of pluripotent stem cells (PSCs). Laminin-521 is a natural component of the stem cell niche *in vivo*. rhLaminin-521 recapitulates a natural environment for maintenance of self-renewal, normal morphology, pluripotency, and karyotype of PSCs cultured in chemically defined, feeder-free, and xeno-free stem cell culture media such as Essential 8™ Medium. Furthermore, rhLaminin-521 supports cell health across the stem cell workflow, enabling improved reprogramming efficiency, efficient passaging of PSCs as a single cell suspension in the absence of inhibitors of apoptosis, as well as efficient transfer of existing feeder-dependent PSC cultures to feeder-free conditions.

Product	Catalog no.	Amount	Storage	Shelf life**
rhLaminin-521	A29248	100 µg*	–30°C to –10°C	2 years from date of receipt
	A29249	1 mg = 100 µg × 10		

*Also available as a kit with Essential 8™ Medium, Essential 8™ Adaption Kit (Cat. no. A25935)

** Shelf life duration is determined from Date of Receipt when stored at recommended storage condition.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

- Thaw rhLaminin-521 slowly at 2ºC to 8ºC. Avoid extended exposure of protein to ambient temperatures. For long coating procedures the laminin stock solution should be kept on ice.
- Once thawed, rhLaminin-521 stock is stable for up to 3 months when stored at 2ºC to 8ºC.
- Divide thawed rhLaminin-521 into usage-size aliquots and store in a non-frost-free freezer at –30ºC to –10ºC. Avoid repeated freeze-thaw cycles.
- Plates can be coated in advance of experiments, parafilm sealed, and stored at 2°C to 8°C under aseptic conditions for up to 2 weeks. Do not allow the culture surface to dry.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture conditions

Culture type: Adherent feeder-free

Substrate: rhLaminin-521

Diluent: DPBS, calcium, magnesium (Cat. no. 14040)

Recommended media: Essential 8™ Medium (Cat. no. A1517001).

Recommended passaging reagents: Cells cultured in Essential 8™ Medium on rhLaminin-521-coated vessels should be passaged using TrypLE™ Select (Cat. no. 12563). Alternatively, passage cells using Versene solution (Cat. no. 15040), a 0.48 mM EDTA solution in PBS.

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Working concentration

The optimal working concentration of rhLaminin-521 is cell line dependent and must be determined empirically. We recommend using an initial coating concentration of 0.5 µg/cm² on the culture surface. Prior to coating culture vessels, calculate the working concentration according to the formula below and dilute the stock appropriately. Refer to Table 1 for culture surface area and required coating volumes.

$$Working\ conc. = Coating\ conc. \times \frac{Culture\ surface\ area}{Vol. required\ for\ surface\ area}$$

$$Dilution\ factor = \frac{Stock\ concentration\ (100\ \frac{\mu g}{mL})}{Working\ concentration}$$

For example, to coat a 6-well plate at a coating concentration of 0.5 µg/cm², you will need to prepare 12 mL of diluted rhLaminin-521 solution (10 cm²/well surface area and 2 mL of diluted rhLaminin-521/well; see Table 1) at the following working concentration:

$$Working\ concentration = 0.5\ \frac{\mu g}{cm^2} \times \frac{10\ cm^2}{2\ mL} = 2.5\ \frac{\mu g}{mL}$$

$$Dilution\ factor = \frac{100\ \mu g/\ mL}{2.5\ \mu g/mL} = 40X\ (i.e., 1{:}40\ dilution)$$

Coat culture vessels with rhLaminin-521

Instructions for coating a 6-well culture plate with rhLaminin-521 at a coating concentration of 0.5 µg/cm² are provided below. For volumes used in other culture vessels, refer to Table 1. To calculate the working concentration of rhLaminin-521 used with other coating concentrations and to determine the appropriate dilution factor, use the equations above.

1. Upon receipt, thaw the vial of rhLaminin-521 slowly at 2°C to 8°C, mix by gentle trituration, and prepare usage size aliquots in polypropylene tubes. Freeze aliquots at –30°C to –10°C or store aliquots at 2°C to 8°C for up to 3 months.
2. To coat the wells of a 6-well plate, add 300 µL aliquot of rhLaminin-521 into a 15-mL conical tube containing 12 mL of sterile DPBS containing calcium and magnesium (Cat. No. 14040). Gently resuspend by pipetting the rhLaminin-521 dilution up and down.
   Note: This results in a working concentration of 2.5 µg/mL (i.e., a 1:40 dilution).
3. Add 2 mL of the diluted rhLaminin-521 solution to each well of a 6-well plate (refer to Table 1 for the recommended volumes for other culture vessels). When used to coat a 6-well plate (10 cm²/well) at 2 mL/well, the final coating concentration will be 0.5 µg/cm².
4. Incubate the plates in a 37°C, 5% CO_2 for 2 hours for efficient coating.
   Note: Alternatively, the plate can be coated at 2°C to 8°C overnight. Do not allow the culture vessel to dry. Prior to use, pre-warm the culture vessel to room temperature.
5. Aspirate the rhLaminin-521 solution and discard. It is not necessary to rinse off the culture vessel after the removal of rhLaminin-521. Cells can be passaged directly onto the rhLaminin-521-coated culture vessels.

Recover single cell passaged human pluripotent stem cells onto rhLaminin-521 coated culture vessels

1. Coat culture vessels with rhLaminin-521 per instructions.
2. Pre-warm the required volume of TrypLE™ Select dissociation reagent in a 37°C waterbath.
3. Aspirate the spent medium from the culture vessel.
4. Rinse the vessel once with recommended volume of Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium (see Table 2).
5. Add the recommended volume of pre-warmed TrypLE™ Select Enzyme (see Table 2).
6. Incubate the vessel at 37°C, 5% CO_2 for 5 minutes.
   Note: Avoid extended incubation of PSCs with dissociation reagents to minimize cellular damage.
7. Gently pipette the cells up and down 5–10 times to generate a single cell suspension.
8. Transfer the cell suspension to a conical tube containing the recommended volume of Essential 8™ Medium to dilute the TrypLE™ Select Enzyme (see Table 2).
9. Centrifuge the PSCs at 200 × *g* for 4 minutes.
10. Discard the supernatant, flick the tube 3–5 times to loosen the pellet, and resuspend the cells by pipetting up and down 5–10 times in the recommended volume of Essential 8™ Medium (see Table 2).
11. Determine the viable cell density and percent viability using a Countess™ Automated Cell Counter or similar automated or manual method.
12. Adjust the concentration of the cell suspension using Essential 8™ Medium to achieve the cell seeding density recommended for your culture vessel (see Table 3).
    Note: Cell seeding densities are cell line dependent and thus may need to be optimized for your cell line.
13. Transfer the cell suspension to the culture vessel pre-coated with rhLaminin-521. Move the vessel in several quick back-and-forth and side-to-side motions to disperse the cells across its surface.
14. Incubate the cells in the recommended cell culture environment.
15. Cells cultured in Essential 8™ Medium must be fed daily.
16. Cells should be passaged once reaching 60%–85% confluency to maintain optimum cell health of cultures.

Table 1 rhLaminin-521 Coating Reagent volumes (per well or per dish)

Culture vessel (surface area)	Volume of diluted rhLaminin-521 solution
6-well (10 cm²)	2 mL
12-well (4 cm²)	0.8 mL
24-well (2 cm²)	0.4 mL
35-mm (10 cm²)	2 mL
60-mm (20 cm²)	4 mL
100-mm (60 cm²)	12 mL

Table 2 Passaging and culture reagent volumes (per well or per dish)

Culture vessel (surface area)	DPBS for wash	TrypLE™ Select	Essential 8™ Medium*	Essential 8™ Medium**
6-well (10 cm²)	2 mL	1 mL	3 mL	2 mL
12-well (4 cm²)	1 mL	0.4 mL	1.2 mL	1 mL
24-well (2 cm²)	0.5 mL	0.2 mL	0.6 mL	0.5 mL
35-mm (10 cm²)	2 mL	1 mL	3 mL	2 mL
60-mm (20 cm²)	4 mL	2 mL	6 mL	4 mL
100-mm (60 cm²)	12 mL	6 mL	18 mL	12 mL

*For neutralization **For resuspension

Table 3 Recommended cell seeding densities and volumes of medium for plating (per well or per dish)

Culture vessel (surface area)	Number of viable cells added*		Essential 8™ Medium**
	12,500 cells/cm²	25,000 cells/cm²	
6-well (10 cm²)	125,000	250,000	2 mL
12-well (4 cm²)	50,000	100,000	1 mL
24-well (2 cm²)	25,000	50,000	0.5 mL
35-mm (10 cm²)	125,000	250,000	2 mL
60-mm (20 cm²)	250,000	500,000	4 mL
100-mm (60 cm²)	750,000	1,500,000	12 mL

*Time to confluency is 4–5 days for a 12,500 cells/cm² seeding density and 3–4 days for a 25,000 cells/cm² seeding density. **For resuspension

Related products

Product	Cat. no.
DPBS, calcium, magnesium	14040
Essential 8™ Medium	A1517001
UltraPure™ 0.5 M EDTA, pH 8.0	15575
Versene Solution	15040
TrypLE™ Select Enzyme (1X), no phenol red	12563
DPBS, no calcium, no magnesium	14190
CytoTune™-iPS 2.0 Kit	A16517

Explanation of symbols and warnings

Symbol	Meaning
⚠	Caution, consult accompanying documents
(thermometer)	Temperature Limitation
(book with i)	Consult instructions for use
Read SDS	Read Safety Data Sheet
(factory)	Manufacturer
LOT	Batch Code
REF	Catalog number

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