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by Thermo Fisher Scientific

Essential 8™ Adaptation Kit

Description

rhLaminin-521 is a recombinant human protein that provides a defined surface for feeder-free culture of human pluripotent stem cells (PSCs). rhLaminin-521 provides optimal PSC survival following feeder-dependent to feeder-free transitions when used in conjunction with Essential 8™ Medium.

Product*	Catalog no.	Amount	Storage	Shelf life
Essential 8™ Adaptation Kit contains:	**A25935**	**1 Kit**	See below	See below
Essential 8™ Basal Medium Essential 8™ Supplement (50X)	A1517001	500 mL 10 mL	2°C to 8°C. Protect from light. –20°C to –5°C. Protect from light.	1 year**
rhLaminin-521	A29248	100 µg	–30°C to –10°C	2 years***

*Essential 8™ Medium (Cat. no. A1517001) and rhLaminin-521 (Cat. no. A29248) are also sold separately.

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

*** 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 reagent: TrypLE™ Select (Cat. no. 12563)

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 60-mm dish at a coating concentration of 0.5 µg/cm², you will need to prepare 4 mL of diluted rhLaminin-521 solution (20 cm²/dish surface area and 4 mL of diluted rhLaminin-521/dish; see Table 1) at the following working concentration:

$$Working\ conc. = 0.5\ \frac{\mu g}{cm^2} \times \frac{20\ cm^2}{4\ mL} = 2.5\ \frac{\mu g}{mL}$$

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

Coat culture vessels with rhLaminin-521

Instructions for coating a 60-mm culture dish 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 a 60-mm dish, add 100 µL aliquot of rhLaminin-521 into a 15-mL conical tube containing 4 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 the diluted rhLaminin-521 solution to the 60-mm dish (refer to Table 1 for the recommended volumes for other culture vessels). When used to coat a 60-mm dish (20 cm²) at 4 mL/well, the final coating concentration will be 0.5 µg/cm².
4. Incubate the plates in a 37°C, 5% CO_2 incubator 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 for at least 1 hour.
5. Immediately prior to plating of cells, 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.

Adapt cells to feeder-free culture in Essential 8™ Medium

Follow the instructions below to adapt feeder-dependent PSC cultures to feeder-free conditions in Essential 8™ Medium on rhLaminin-521-coated culture vessels. The volumes given in the procedure are for 60-mm culture dishes. For volumes used in other culture vessels, refer to Table 2.

1. When the feeder-dependent cultures reach passaging confluency (60%–85% confluent with round colonies that are not overcrowded), the cells are ready for adaptation to feeder-free culture conditions.
2. Coat culture vessels with rhLaminin-521 per instructions noted above.
3. Prepare a 1 mg/mL Collagenase Type IV solution in DMEM/F12 with GlutaMAX™ Supplement and filter sterilize using a 0.2-um filter unit.
4. Aspirate the spent medium from the culture vessel.
5. Rinse the vessel once with 4 mL of Dulbecco's Phosphate Buffered Saline (DPBS) without calcium or magnesium.
6. Add 2 mL of 1 mg/mL Collagenase Type IV, pre-warmed to 37ºC.
7. Incubate the vessel for ~45 minutes in a 37°C, 5% CO_2 incubator.
   Note: Stop the incubation when the edges of the colonies begin to curl from the plate. Do not over-incubate.
8. Add 2 mL of Essential 8™ Medium and gently dislodge the colonies from the plate by washing off colonies with a 5-mL serological pipette. Repeat trituration until the desired cluster size is achieved.
9. Transfer the suspended colony clusters into a 15-mL conical tube.
10. Add 2 mL of Essential 8™ Medium to dislodge the remaining colonies and transfer them to the 15-mL conical tube.
11. Let the colony fragments sediment to the bottom of the 15-mL conical tube for 5 minutes by gravity.
12. Discard the supernatant, add 4 mL of Essential 8™ Medium, and gently resuspend the sedimented colony fragment by pipetting up and down 2 times.
13. Gravity sediment the clusters for 2–5 minutes.
14. While the colony fragments are sedimenting, aspirate the matrix solution from the freshly prepared rhLaminin-521 coated 60-mm dish and add 4 mL of Essential 8™ Medium.
15. Aspirate the supernatant and resuspend the sedimented PSC clusters by gently pipetting them up and down 2 times in 4 mL Essential 8™ Medium, taking care not to break them down further.
16. Distribute 1 mL of the resuspended PSC clusters into the rhLaminin-521 pre-coated dish. Move the vessel in several quick back-and-forth and side-to-side motions to disperse the cells across its surface.
17. Incubate the cells in a 37°C, 5% CO_2 incubator and passage them when they are 60%–85% confluent to maintain optimal cell health.
    Note: Cells cultured in Essential 8™ Medium must be fed daily.

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 cm^2)	2 mL
12-well (4 cm^2)	0.8 mL
24-well (2 cm^2)	0.4 mL
35-mm (10 cm^2)	2 mL
60-mm (20 cm^2)	4 mL
100-mm (60 cm^2)	12 mL

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

Culture vessel (surface area)	DPBS for wash	1 mg/mL Collagenase IV	Essential 8™ Medium*	Essential 8™ Medium**
6-well (10 cm^2)	2 mL	1 mL	1 mL	2 mL
12-well (4 cm^2)	1 mL	0.4 mL	0.4 mL	8 mL
24-well (2 cm^2)	0.5 mL	0.2 mL	0.2 mL	0.4 mL
35-mm (10 cm^2)	2 mL	1 mL	1 mL	2 mL
60-mm (20 cm^2)	4 mL	2 mL	2 mL	4 mL
100-mm (60 cm^2)	12 mL	6 mL	6 mL	12 mL

*For initial resuspension and for wash off. **For final two resuspensions.

Note: Split ratios may need to be optimized depending on the cell line and the percentage confluency of PSCs at the time of harvest.

Related products

Product	Cat. no.
DPBS, calcium, magnesium	14040
Essential 8™ Medium	A1517001
rhLaminin-521	A29248
TrypLE™ Select Enzyme (1X), no phenol red	12563
DPBS, no calcium, no magnesium	17104
Collagenase, Type IV, Powder	A16517
DMEM/F12, GlutaMAX™ Supplement	10565

Explanation of symbols and warnings

				
Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
				
Batch Code	Catalog number	Manufacturer	Read Safety Data Sheet	

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