

Human Laminin 521 Protein (PG)



Cat. No. LMN-HM522

Description

Source	Mammalian cells
Purity	≥95%
Concentration	0.1 mg/ml
Endotoxin	<1 EU/ml
Mycoplasma	Negative
Animal origin	Animal origin-free

Formulation and Storage

Formulation	PBS (pH 7.4)
Storage	Valid for 2 years from date of receipt when stored at -20°C to -80°C. Thawed, undiluted stock is stable for 3 months when stored at 2-8°C under aseptic conditions.

Operation Guide

1. Thaw Laminin 521 slowly at 2°C to 8°C. For prolonged operation, the product should be placed on ice. If longer storage is needed, we recommend dividing the thawed stock solution in smaller working aliquots and to store frozen.

NOTE: Avoid repeated freeze-thaw cycles.

2. Dilute the product in DPBS (with Ca²⁺ and Mg²⁺) to the final concentration of 5-10 µg/ml. When culturing cells with this product for the first time, higher coating concentrations are recommended for the first few cell passages until the cells have adapted to the matrix.

NOTE: The required concentration of Laminin 521 should be cell-dependent and optimized for each application. we recommend using an initial coating concentration of 0.5 µg/cm² on the culture surface and can operate according to the following formula:

Working conc. = Coating conc. × (Culture surface area / Vol. required for surface area)

Dilution factor = Stock concentration / Working concentration

(The Coating conc. and Vol. required for surface area depends on different experimental needs)

3. Mix solution gently, do not vortex.

4. Immediately add the diluted Laminin 521 to the cultureware. Recommended coating volumes are as follows:

Cultureware	Volume of diluted Laminin 521
6-well plate	1 ml/well
12-well plate	0.5 ml/well
24-well plate	0.2 ml/well
T-25 cm ² flask	2.5 ml/flask
T-75 cm ² flask	7.5 ml/flask
35 mm dish	0.8 ml
60 mm dish	2 ml
100 mm dish	5.5 ml

NOTE: The above volume is calculated according to the concentration of 5 µg/ml. The bottom area is subject to the actual use.

5. Gently rock the cultureware back and forth to spread the Laminin 521 solution evenly across the entire surface.

6. Seal the cultureware to prevent evaporation of Laminin 521 solution, then incubate at 2-8°C overnight. If more rapid coating is required, incubate at 37°C for at least 2h before use. The cultureware can be coated in advance of experiments, sealed and stored at 2-8°C under aseptic conditions for up to 2-4 weeks.

NOTE: Do not allow the culture surface to dry as that will inactivate the matrix coating.

7. Aspirate Laminin 521 when cells are ready to be plated.

NOTE: The coating does not require washing before use.

Background

Laminin 521, also known as LN521 or LN-11, is a heterotrimeric glycoprotein composed of one alpha 5, one beta 2, and one gamma 1 chain. Laminin 521 is a natural component of the stem cell niche in vivo and it is prominently expressed in embryonic BM and glomerular BM. A cell culture condition that contains LN-521 allows long-term maintenance of the stemness of pluripotent stem cells (PSCs), ESCs, and hiPSCs under chemically defined and xeno-free matrices without apoptosis inhibitors.

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References

- [1] Laminins in Cellular Differentiation. Trends Cell Biol. 2019 Dec;29(12):987-1000.
- [2] Monolayer culturing and cloning of human pluripotent stem cells on laminin-521-based matrices under xeno-free and chemically defined conditions. Nat Protoc. 2014 Oct;9(10):2354-68.
- [3] A defined xeno-free and feeder-free culture system for the derivation, expansion and direct differentiation of transgene-free patient-specific induced pluripotent stem cells. Biomaterials. 2014 Mar;35(9):2816-26.
- [4] Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Nat Commun. 2014;5:3195.