60 Hickory Drive
Waltham, MA 02451
United States

Tel: (617) 665-7333
support@kactusbio.us
kactusbio.com

Laminin 521, Preclinical-Grade
#LMN-HM522

Product Component	Sizes
Laminin 521 (0.1mg/mL)	100µg, 500µg, 1mg

PRODUCT INFORMATION

Property	Description
Storage/Transport	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.
Form	Liquid
Source	Recombinant protein expressed in a human cell line
Concentration	0.1mg/mL

PRODUCT DESCRIPTION
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.

APPLICATIONS
- Supports stable self-renewal of hPSCs
- Improved recovery and survival following passage of singularized PSCs in the absence of ROCK inhibitors
- MSC and other stem cell culture
- Maintain the differentiation potential of cells in long-term culture

QUALITY CONTROL

Assay	Criteria
Appearance	Clear, buffered solution with a pH of 7.4 with PBS
Endotoxin	< 10EU/mg
Purity	≥ 95%
TSE/BSE	No materials of animal origin; free of TSE/BSE

RECOMMENDED PROTOCOL
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 into smaller working aliquots and storing frozen. Avoid repeated freeze-thaw cycles.
2. Dilute the product in DPBS (with Ca^{2+} and Mg^{2+}) to a final concentration of 5-10µg/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 can be cell-dependent and should be optimized for each application. We recommend using an initial coating concentration of 0.5µg/cm^2 on the culture surface. Working concentration and dilution factor are calculated according to the following formulas:

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$$\text{Working Concentration} = \text{Coating Concentration} \times \frac{\text{Culture Surface Area}}{\text{Volume Required for Surface Area}}$$

$$\text{Dilution Factor} = \frac{\text{Stock Concentration}}{\text{Working Concentration}}$$

3. Mix solution gently. Do not vortex.
4. Immediately add the diluted Laminin 521 to cultureware. Recommended coating volumes are as follows:

Cultureware	**Volume of Diluted (5µg/mL) Laminin 521**
6-well plate	1 mL/well
12-well plate	0.5 mL/well
24-well plate	0.2 mL/well
T-25 flask	2.5 mL/flask
T-75 flask	7.5 mL/flask
35mm dish	0.8 mL
60mm dish	2 mL
100mm dish	5.5 mL

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.

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.