

BioLamina develops, manufactures and distributes cell culture reagents that make it possible to culture pluripotent stem cells, adult stem cells and tissue-specific cells in a cell-specific and physiologically relevant environment. In vivo, laminins are key proteins in the basement membrane that underlie all epithelia and endothelia and surround individual cells, with essential roles in the regulation of many cellular functions, such as adhesion, differentiation, migration, phenotype stability, and resistance to apoptosis. BioLamina's human recombinant laminin cell culture matrices, Biolaminin, are chemically defined and animal origin-free and makes cell culture easy and standardized.

## Transition protocol

### IMPORTANT NOTES

- All procedures should be done under sterile conditions using aseptic techniques
- Avoid prolonged exposure of the protein to ambient temperatures
- Repeated freeze/thaw should be avoided
- The Biolaminin stock solution is stable for 3 years when stored at -20°C to -80°C
- Thawed, undiluted Biolaminin stock is stable for at least 3 months when stored at +2°C to +8°C under aseptic conditions
- For your convenience, coated plates can be kept for up to 4 weeks when

### 1 Slowly thaw the Biolaminin stock solution at +2°C to +8°C before use.

Thawed, undiluted Biolaminin stock is stable for at least 3 months when stored at +2°C to +8°C under aseptic conditions. Repeated freeze-thaw cycles should be avoided. For longer storage needs, we recommend dividing the thawed stock solution in smaller working aliquots and to store frozen. Frozen stock can be stored up to three years in -20°C to -80°C.

### 2 Calculate the concentration and the amount of coating solution needed for the experiment. The MX and CTG matrix generally should be used in a slightly higher concentration (15-20 µg/mL) compared to the LN matrix (5-10 µg/mL). Once the cells are adapted, a lower coating concentration often can be used but should be optimized empirically for each cell line. Guidelines for surface coating calculations can be found in the table below.

- A reduced coating concentration might affect the proliferation rate, extending the culture time with about 1 day. Make sure the coating concentration is high enough to support even cell growth.
- When adjusting the coating concentration, it is important to maintain the coating volume recommended in the table below. Make sure that the volume covers the entire culture surface as an uncoated surface will not support cell growth.
- When culturing cells on the Biolaminin matrix for the first time, a higher coating concentration (10 µg/mL for LN521 and 20 µg/mL for MX521 and CT521) is recommended for the first few cell passages. Some cell lines might need an adaptation period and a higher coating concentration is then recommended for the first few passages. Once the cells are adapted to the Biolaminin matrix, the coating concentration usually can be reduced. The coating should be optimized empirically for each cell line and cell type.

# Culturing human ES and IPS cells

On human recombinant Biolaminin® 521

INSTRUCTIONS  
FOR USE 001

- 3 Gently invert the vial to mix the Biolaminin stock solution. Do not vortex as this may cause fragmentation.**
- 4 Dilute the Biolaminin stock solution with 1×DPBS (Ca<sup>++</sup>/Mg<sup>++</sup>) and add the solution to the cultureware of choice. There is no need to pre-treat the cultureware. Make sure the entire surface is covered by the laminin coating solution. An uncoated surface will not support cell growth. Recommended coating volumes for different cultureware formats can be found in the table below.**
  - DPBS with Ca<sup>2+</sup> and Mg<sup>2+</sup> should be used since divalent cations are important for the protein structure and function.
  - The laminin matrices work well with most commercial cultureware brands (e.g. Falcon, Sarstedt, Corning).
  - The laminin matrix can easily be used for coating of glass. Overnight coating at +2°C to +8°C is recommended for a more reliable coating.
- 5 Seal the plate (e.g. with Parafilm) to prevent evaporation and contamination. Incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours.**
  - Do not allow the coated surface to dehydrate as that will inactivate the Biolaminin coating.
  - For your convenience, the coated plates can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C. Extra 1×DPBS (Ca<sup>++</sup>/Mg<sup>++</sup>) might have to be added after 1-2 weeks to prevent the plate from drying out.

## Guidelines for surface coating calculations

Cultureware	Coating concentration (Ug/MI)	Coating concentration (Ug/cm <sup>2</sup> )	Coating solution		Total coating solution volume
			Laminin stock	1×DBPS (Ca <sup>++</sup> /Mg <sup>++</sup> )	
6-well	5	0.45	50 uL/well	950 uL/well	1000 uL/well
12-well	5	0.51	25 uL/well	475 uL/well	500 uL/well
24-well	5	0.55	15 uL/well	285 uL/well	300 uL/well
48-well	5	0.49	7.5 uL/well	142.5 uL/well	150 uL/well
96-well	5	0.46	3.5 uL/well	66.5 uL/well	70 uL/well
T-25cm <sup>2</sup> flask	5	0.55	150 uL/flask	2850 uL/flask	3000 uL/flask
T-75cm <sup>2</sup> flask	5	0.51	400 uL/flask	7600 uL/flask	8000 uL/flask

\* Calculations based on the entire surface area coated.

## BioLamina's human recombinant laminin products



**Biolaminin 521 CTG**  
CT521

**Biolaminin 511 LN**  
LN511

**Biolaminin 221 LN**  
LN221

**Biolaminin 521 MX**  
MX521

**Biolaminin 421 LN**  
LN421

**Biolaminin 211 LN**  
LN211

**Biolaminin 521 LN**  
LN521

**Biolaminin 411 LN**  
LN411

**Biolaminin 121 LN**  
LN121

**Biolaminin 332 LN**  
LN332

**Biolaminin 111 LN**  
LN111