BioLamina
REVOLUTIONIZING CELL CULTURE

INSTRUCTIONS FOR USE 001

COATING PLATES

WITH HUMAN RECOMBINANT LAMININS FOR CELL CULTURE

BIOLAMINA DEVELOPS, MANUFACTURES AND DISTRIBUTES CELL CULTURE REAGENTS THAT MAKES 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 REGULATION OF MANY CELLULAR FUNCTIONS, SUCH AS ADHESION, DIFFERENTIATION, MIGRATION, PHENOTYPE STABILITY, AND RESISTANCE TO APOPTOSIS. BIOLAMINA'S HUMAN RECOMBINANT LAMININ CELL CULTURE MATRICES ARE CHEMICALLY DEFINED AND XENO-FREE AND MAKES CELL CULTURE EASY, STANDARDIZED AND MORE AUTHENTIC.

IMPORTANT NOTES

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

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COATING PROTOCOL

1. Slowly thaw the laminin stock solution at +2°C to +8°C before use.
   - *Repeated freeze-thaw cycles should be avoided. Thawed, undiluted laminin stock is stable for at least 3 months when stored at +2°C to +8°C under aseptic conditions.*
2. Calculate the amount of coating solution needed for the experiment. An initial coating concentration of 5 µg/mL is recommended for the first few cell passages. Once the cells are adapted, a lower coating concentration often can be used (down to 1 ug/mL) but should be optimized empirically for each cell line. Guidelines for surface coating calculations can be found in the table below.
   - *Lowering the 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 an even cell growth.*
   - *When using the laminin matrices for the first time, some cell lines might need some adaptation and a higher coating concentration (up to 10 µg/mL) is then recommended for a few passages before the coating concentration can be reduced.*
   - *Some primary cell types might need a higher coating concentration (10-20 µg/mL). The coating should be optimized empirically for each cell type.*
3. Dilute the laminin stock solution with 1xDPBS (Ca^{++}/Mg^{++}) and add the solution to the cultureware of choice.
   - *DPBS with Ca^{2+} and Mg^{2+} 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.*

ORIGINAL

4. Make sure the entire surface is covered by the laminin coating solution. Uncoated surface will not support cell growth. Recommended coating volumes for different cultureware formats can be found in the table below.

5. The cultureware must be sealed (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 laminin 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 1xDPBS (Ca^{++}/Mg^{++}) 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/mL)	COATING CONCENTRATION (ug/cm^2)*	COATING SOLUTION		TOTAL COATING SOLUTION VOLUME
			LAMININ STOCK**	1xDPBS (Ca^{++}/Mg^{++})	
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^2 flask	5	0.55	150 uL/flask	2850 uL/flask	3000 uL/flask
T-75cm^2 flask	5	0.51	400 uL/flask	7600 uL/flask	8000 uL/flask

* Calculations based on the entire surface area coated.

**Calculations in the guideline are based on laminin stock concentration of 100 ug/mL which is the concentration of all laminin isoforms except for laminin-332 (LN332). Please note that LN-332 has a stock concentration of 60 ug/mL, hence, the laminin stock volume have to be recalculated accordingly.

BIOLAMINA'S HUMAN RECOMBINANT LAMININ PRODUCTS

LN521
rhLaminin-521

LN511
rhLaminin-511

LN421
rhLaminin-421

LN411
rhLaminin-411

LN332
rhLaminin-332

LN221
rhLaminin-221

LN211
rhLaminin-211

LN121
rhLaminin-121

LN111
rhLaminin-111

FOR MORE INFORMATION ABOUT HOW OUR LAMININ CELL CULTURE MATRICES CAN BE USED FOR YOUR SPECIFIC CELL APPLICATION, PLEASE VISIT BIOLAMINA'S SCIENCE ROOM

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