BioLamina
REVOLUTIONIZING CELL CULTURE

COATING PLATES

WITH HUMAN RECOMBINANT LAMININS FOR CELL CULTURE

INSTRUCTIONS FOR USE **BL001**

HUMAN RECOMBINANT LAMININS PROVIDE A BIORELEVANT, CHEMICALLY DEFINED AND XENO-FREE CELL CULTURE SYSTEM

COATING PROTOCOL

- Slowly thaw recombinant laminins at +2°C to +8°C before use.
- Dilute the thawed laminin stock solution with 1xDPBS (Ca^{++}/Mg^{++}) and add the diluted laminin solution to the cultureware of choice. When culturing the cells on the laminin matrix for the first time, a coating concentration of 10 µg/mL is recommended for the first few passages. Once the cells are adapted, a lower coating concentration generally can be used which should be optimized empirically for each cell line. Guidelines for surface coating calculations can be found in the tables below.

  DPBS with Ca^{2+} and Mg^{2+} should be used since divalent cations are important for the protein structure and function.

  The laminin coating is not optimized for certain plastics and work well with most commercial culture-ware brands (e.g. Falcon, Sarstedt, Corning).

  Laminin can easily be used for coating of glass. Coat glassware in the same way as for plastic culture-ware. Overnight coating at +2°C to +8°C is recommended for a more reliable coating. Seal the coated glassware to avoid evaporation.
- 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 tables below.
- Incubate at +2°C to +8°C overnight. Seal the cultureware (e.g. with Parafilm®) to prevent evaporation and contamination.

  Overnight coating at +2°C to +8°C is strongly recommended since that provides a more reliable coating. If a more rapid coating is required, incubate at +37°C for 2 hours.

  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 to prevent the plate from drying out.
- When using the coated plate for cell culture, simply aspirate the coating liquid and add cell culture medium of choice.

  The laminin coating does not require washing before use.

  Do not let the laminin wells dry out at any point of the procedure since that will inactivate the matrix.

IMPORTANT NOTES

- **All procedures should be done under sterile conditions using aseptic techniques.**
- **Avoid long exposure of the protein to ambient temperatures.**
- **The laminin stock solution is stable for 2 years when stored at -20°C.**
- **Repeated freeze/thaw should be avoided.**
- **Thawed 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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ORIGINAL

ADAPTATION

When using the laminin matrix for the first time, the cells might need some adaptation, hence a higher coating concentration is recommended for the first few passages. Guideline for surface coating calculations can be found in the table 1 below.

Table 1

CULTUREWARE	COATING CONCENTRATION (ug/mL)	COATING CONCENTRATION (ug/cm²)*	COATING SOLUTION		TOTAL COATING SOLUTION VOLUME
			LAMININ STOCK**	1xDPBS (Ca^{++}/Mg^{++})	
6-well	10	0.90	100 uL/well	900 uL/well	1000 uL/well
12-well	10	1.02	50 uL/well	450 uL/well	500 uL/well
24-well	10	1.09	30 uL/well	270 uL/well	300 uL/well
48-well	10	0.98	15 uL/well	135 uL/well	150 uL/well
96-well	10	0.93	7 uL/well	63 uL/well	70 uL/well
T-25cm² flask	10	1.09	300 uL/flask	2700 uL/flask	3000 uL/flask
T-75cm² flask	10	1.02	800 uL/flask	7200 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 LN-332. Please not the that LN-332 has a stock concentration of 60 ug/mL, hence, the the laminin stock volume have to be recalculated accordingly.

ROUTINE USE

Once the cells are adapted to the laminin matrix a lower coating concentration usually can be used. The optimal coating concentration should be optimized empirically for specific laminin isoform and cell line. Guideline for surface coating calculations can be found in the table 2 below.

Table 2

CULTUREWARE	COATING CONCENTRATION (ug/mL)	COATING CONCENTRATION (ug/cm²)*	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-25cm² flask	5	0.55	150 uL/flask	2850 uL/flask	3000 uL/flask
T-75cm² 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 LN-332. Please not the that LN-332 has a stock concentration of 60 ug/mL, hence, the the laminin stock volume have to be recalculated accordingly.

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BioLamina
REVOLUTIONIZING CELL CULTURE

INSTRUCTIONS FOR USE BL002

CULTURING ES AND iPS CELLS
ON HUMAN RECOMBINANT LAMININ-511

BIORELEVANT HUMAN RECOMBINANT LN-511™ PROVIDES A DEFINED AND XENO-FREE COATING FOR MOUSE PSCS WHERE ADDITION OF DIFFERENTIATION INHIBITORS, SUCH AS LEUKEMIA INHIBITORY FACTOR (LIF), IS NO LONGER NEEDED

The following protocol is a generic guideline that might require optimization for best results. The protocol is for single cell passage of mouse pluripotent stem cells but colony passaging can also be used if applicable. When transferring your cells from another feeder-free matrix (e.g. Matrigel) we recommend you to start with a smaller well format and a higher seeding density for the first number of passages to let the cells adapt to the laminin matrix. When moving your cells from feeders to LN-511, follow the protocol in **INSTRUCTIONS FOR USE BL004**.

PASSAGING PROTOCOL

- Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C. Volumes listed are for 6-well plates and should be adjusted accordingly if using alternative size of cultureware.
- Coat a new cultureware in advance with the laminin solution as described in **INSTRUCTIONS FOR USE BL001.**
- Carefully remove the laminin coating solution without disturbing the coated surface and immediately add 1 mL fresh media to each well and let equilibrate at +37°C, 5% CO_2.
  The laminin coating does not require washing before use.

  Do not let the laminin wells dry out at any point of the procedure, as this will inactivate the laminin matrix.
- Aspirate the medium from the cells and wash gently with 1xDPBS (Ca^{--}/Mg^{--}).
  DPBS without Ca^{2+} and Mg^{2+} should be used since divalent cations have negative effect on some dissociating enzymes.

IMPORTANT NOTES

- **All procedures should be done under sterile conditions using aseptic techniques.**
- **The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.**
- **The laminin stock solution is stable for 2 years when stored at -20°C.**
- **Repeated freeze/thaw should be avoided.**
- **Thawed 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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- Add 1 mL/well of dissociation enzyme of choice (e.g. TrypLE™) and incubate at +37°C for 3-5 minutes.
  The incubation time is cell line dependent and also depends on the solution used to dissociate the cells. We recommend enzyme dissociation for reliable single cell suspension passage. However, if applicable, enzyme-free dissociation, such as EDTA, can also be used. Stem cells are sensitive and too long exposure to dissociation enzymes or too much mechanical force may result in low cell viability.
- Gently aspirate the dissociation solution and add 1 mL/well of pre-warmed fresh medium. Gently pipette up and down 6 -10 times to achieve single-cell suspension.
  Make sure the cells are properly dissociated into single cells by using a microscope. Minimize the mechanical force applied not to cause significant physical damage to the cells.
- Collect the cell suspension in a conical tube.
- Centrifuge at 100 x *g* for 4 minutes at (+15°C to +25°C) and discard the supernatant.
- Resuspend the cell pellet in 1-2 mL/well of fresh, pre-warmed culture medium.
- Count the cell number and seed the cells with an appropriate cell density.
  Optimal seeding densities will vary from one cell line to another and can be determined empirically for your system.

  When moving your cells from another feeder-free matrix (e.g. Matrigel) we recommend you to start with a smaller well format (e.g. 24-well or 48-well format) and a higher seeding density (>100,000 cells/cm^2) for the first number of passages to let the cells adapt to the laminin matrix before increasing the well format and lowering the seeding density.

  When moving your cells from feeders to LN-511, follow the protocol in **INSTRUCTIONS FOR USE BL004**.
- Gently rock the plate to distribute the cells evenly and then place them into an incubator (+37°C, 5% CO_2).
- Feed cells on daily basis until next passage.
  Freshly seeded cells only need a few drops of fresh medium after 24 hours. Perform a complete medium change 48 hours after passaging. Cells are ready to be passaged when the cell culture is ≥60% confluent.

  When using LN-511, colony passage can also be applied, although we recommend single cell passage for robust and easy cell culture.

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