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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INSTRUCTIONS FOR USE **BL003**

CULTURING HUMAN PSCs
ON HUMAN RECOMBINANT LAMININ-521

THE DEFINED AND XENO-FREE HUMAN RECOMBINANT LN-521™ COATING PROVIDES A BIORELEVANT STEM CELL NICHE

The following protocol is for easy single cell passage of human pluripotent stem cells. It is a generic guideline that might require optimization for best results. 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-521, 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** or use 521-To-Go™ pre-coated plates described in **INSTRUCTIONS FOR USE BL007.**
- 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 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^{--}).
  Cells are ready to be passaged when cell culture is ≥60% confluent.

  DPBS without Ca^{2+} and Mg^{2+} should be used since divalent cations have negative effect on some dissociating enzymes.
- 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 and dissociation reagent dependent. We recommend enzymatic dissociation for reliable single cell suspension passage. However, if applicable, enzyme-free dissociation, such as with EDTA, can also be used. Stem cells are sensitive and too long exposure to enzymes or too much mechanical force may result in low cell viability.

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.**
- **LN-521 facilitates long-term self-renewal of hPSC without weekend feeding. Follow the protocol and recommendations in WEEKEND-FREE CULTURE.**
- **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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- 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.
  Culture medium can be determined accordingly by the user for different cell types and applications. We have successfully tested most commercial media brands (e.g. NutriStem™, mTeSR™1, TeSR™2, and Essential 8™).

  If medium transition is needed, gradually increase the ratio of the new medium until the final and complete medium transition. It is not recommended to change both the medium and matrix at the same time. Preferably transfer PSCs to LN-521 before the change of medium since LN-521 supports most commercial media brands.
- Count the cell number and seed the cells with density of 30,000-50,000 cells/cm^2 or with a split ratio of 1:10 to 1:30.
  LN-521 has been shown to support cell survival of as low as 5,000 cells/cm^2, however, 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 to start with a smaller well format (e.g. 24-well or 48-well format) and a higher seeding density (50,000-100,000 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-521, 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. With optimal media conditions and seeding density, most cell lines will reach confluence within 4-6 days and expand 10-25 fold.

  Alternatively, for less work and medium usage, follow the weekend-free protocol for **WEEKEND-FREE CULTURE**.

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INSTRUCTIONS FOR USE **BL004**

CHANGING SUBSTRATES

FROM FEEDERS AND FEEDER-FREE MATRICES TO LN-521

CHANGING SUBSTRATES TO THE LN-521™ STEM CELL MATRIX

FROM FEEDER-FREE MATRICES:
The transfer from feeder-free matrices to LN-521 is often quite straight forward. Coat new plates with LN-521 according to **INSTRUCTIONS FOR USE BL001** and perform single cell passage as described in **INSTRUCTIONS FOR USE BL003**. We recommend to start with a smaller well format (e.g. 24-well or 48-well format) and a higher seeding density (50,000-100,000 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.

FROM FEEDERS:
Usually it is sufficient to collect the pieces of undifferentiated hPSC colonies from the feeder plate, seed them directly on LN-521 (see **INSTRUCTIONS FOR USE BL001** for coating instructions), and when the cells reach about 70% confluence, perform an enzymatic single cell passage for maintenance as described in **INSTRUCTIONS FOR USE BL003**. We recommend to start with a smaller well format (e.g. 96-well or 48-well format) and a higher seeding density (50,000-100,000 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.

Some hPSC lines are more difficult to transfer and will thus need parallel single cell and colony passage for several passages to ensure single cell survival as described below.

IMPORTANT NOTES

- **All procedures should be done under sterile conditions using aseptic techniques.**
- **It is important that the cells transferred to LN-521 are of high quality. Carefully selected undifferentiated colonies only for transfer.**
- **The cells are to be transferred to the LN-521 coated plate as single cells but might require some adaption for a few passages. It may take up to 5 mechanical colony passages before some lines are used to the LN-521 environment and can tolerate enzymatic single cell passage.**
- **Human PSCs survive transfer better if they can efficiently condition the medium. Initially seed 25–30 small aggregates (approx. 100 µm size) per well (96-well plate) in 120–150 µL medium.**
- **Pluripotent stem cells on feeders can be adapted to new media formulations by small additions of the new medium a few days before the complete transition. Culture medium can be determined accordingly by the user. We have successfully tested several different commercial media brands, such as Nutristem™, mTeSR™1, TeSR™2.**
- **Thawed laminin stock solution is stable for at least 3 months when stored at +2-8°C under aseptic conditions.**
- **For your convenience, the coated plates can be kept for up to 4 weeks when stored at +2-8°C under aseptic conditions.**

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STEP 1 - TRANSFER PROTOCOL

- Before start, all solutions used should be aliquoted in sufficient amounts and pre-warmed at +37°C.
- Coat 4-8 wells (depending on quantity of cells for transfer) of a 96-well plate with LN-521 in advance as described in **INSTRUCTIONS FOR USE BL001** or use 521-To-Go™ pre-coated plates (**INSTRUCTIONS FOR USE BL007**).
- 1 hour before a transfer, carefully remove the laminin coating solution without disturbing the coated surface and immediately add 100 µl fresh medium to each well and let equilibrate at +37°C, 5% CO_2.
  - Use the same medium as on the feeder plate. If a medium change is needed, do it after cells have been transferred to LN-521 successfully.
  - The Laminin coating does not require washing before use. Do not let the laminin coated wells dry out at any point of the procedure as this will inactivate the laminin matrix.
- Aspirate the medium from the hPSCs on feeders and wash gently with 1x DPBS (Ca^{--}/Mg^{--}).
- Separate hPSC cell aggregates from the feeder cells using standard methods (e.g. cut and scrape hPSC colonies with a knife) to a size of approx. 100 µm.

  CRITICAL POINTS
  - Do not feed the medium for hPSCs on feeders before the procedure.
  - Choose undifferentiated parts of the colonies only.
  - Do the cutting relatively fast. Keep the LN-521 coated plate at 37 °C and 5% CO_2 while cutting.
- Swirl the feeder plate to collect the aggregates in the middle. Carefully aspirate 20–30 free-floating aggregates with a pipette. Use a microscope if needed.
- Place 20–30 aggregates/well into the prepared LN-521 coated 96-well plate containing 100 µL of pre-warmed medium of choice per well.

  CRITICAL POINTS
  - It is important to have enough amounts of hPSC colonies for each new LN-521 well to be able to efficiently condition the medium. Too few colonies/well may lead to differentiation of the cells.
  - Distribute the aggregates evenly by moving them in different parts of the well.
- Repeat the seeding of 20-30 aggregates in different LN-521 coated wells. Incubate the plate at 37 °C and 5% CO_2.
  - Do not disturb the cells for 48h after plating.
- After 48h, feed the cells daily and monitor the cell growth with a microscope and move to the next step when they are 90-100% confluent.
- Coat 6 wells of a new 96-well plate in advance with LN-521 as described in **INSTRUCTIONS FOR USE BL001**.
- Carefully remove the laminin coating solution without disturbing the coated surface and immediately add 100 µL/well of fresh medium and let equilibrate at +37°C, 5% CO_2.
- Choose the two wells with most confluent and homogeneous cells to perform both conventional single cell passage from one well, and an enzyme-free colony passage from the other well.
  - Perform single cell passage and colony passage in parallel until the cells are successfully adapted to the LN-521 matrix and the cells efficiently can be cultured as single cells.

STEP 2A - SINGLE CELL PASSAGE

- Before start, all solutions used should be aliquoted in sufficient amounts and pre-warmed at +37°C.
- Aspirate the medium from the cells and wash gently with 80 µL/well 1x DPBS (Ca^{--}/Mg^{--}). DPBS without Ca^{2+} and Mg^{2+} should be used since divalent cations have negative effect on some dissociating enzymes.
- Add 80 µL/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. 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 100 µL/well of pre-warmed fresh medium.
- Gently pipette up and down 5-10 times to achieve a single cell suspension. Use 200 µL pipette tips.
  - Mechanical force should be minimized not to cause significant physical damage to the cells.
- Collect the cell suspension in a conical tube and centrifuge at 100 x g for 4 minutes at +15°C to + 25°C and discard the supernatant.
- Resuspend the cell pellet in 100 µL of fresh, pre-warmed culture medium.
- Divide the cell suspension into 3 new LN-521 coated wells of a 96-well plate (a 1:3 split).
  - Plate cells with a higher seeding density (50,000-100,000 cells/cm^2) for the first number of passages to let the cells adapt to the LN-521 matrix before increasing the well format and lowering the seeding density. To increase the accuracy, it is recommended to count the cell number.
- Place the plate into an incubator (+37°C, 5% CO_2).
  - Do not disturb the cells for 48h after plating and after that perform daily feeding of the cells by complete medium change.
  - The cells are ready for passage when the culture is >60% confluent.

STEP 2B - COLONY PASSAGE

- Before start, all solutions used should be aliquoted in sufficient amounts and pre-warmed at +37°C.
- Aspirate the medium from the cells and wash gently with 80 µL/well 1x DPBS (Ca^{--}/Mg^{--}).
- Add 80 µL/well 1x DPBS (Ca^{--}/Mg^{--}) and incubate at +37°C for 5 minutes.
- Gently aspirate the 1x DPBS (Ca^{--}/Mg^{--}) and add 100 µL/well of pre-warmed fresh medium.
  - Gently break the monolayer of cells by scraping with a pipette tip, and create a uniform suspension of aggregates (approx. 100 µm in size) by gentle trituration with a pipette (max 3 times up and down). Use 200 µL pipette tips.
  - Monitor the aggregate suspension under a microscope.
- Divide the aggregate suspension in 3 new LN-521 coated wells of 96-well plate (a 1:3 split).
  - Gently tap the plate to distribute the aggregates evenly.
- Place the plate into an incubator (+37°C, 5% CO_2).
  - Do not disturb the cells for 48h after plating and after that perform daily feeding of the cells by complete medium change
  - The cells are ready for passage as soon as the colonies start to touch each other. To continue the culture beyond this point will result in differentiation.

STEP 3

- Repeat the procedure above until the cells successfully are adapted to single cell passage. Choose the well(s) with the most homogenous cells at all times. Use a microscope to monitor the growth of the cells.
- When the cells are successfully adapted to single cell passage, perform conventional single cell passaging as described in **INSTRUCTIONS FOR USE BL003**. Seed a higher cell density (50,000-100,000 cells/cm^2) and in smaller well formats (96 or 48-well format) for the initial passages. Once the cells are adapted to LN-521 the seeding density can gradually be lowered and well format increased.

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CELL CULTURE ON BIOLOGICALLY RELEVANT LAMININ

Cell culture of primary cells and stem cells is reliable and robust when growing your cells on the natural human recombinant laminin that match your cell of interest. Almost all cells grow on specific laminins in the human body and as these laminins are now available as recombinant laminins this makes it possible to culture cells in a physiologically relevant environment.

BioLamina offers defined cell culture environments that enable cell culture of pluripotent stem cells, adult stem cells and tissue-specific cells. Cell culture of motor neurons and dopaminergic neurons, neural stem cells, cardiomyocytes, endothelial cells, pancreatic beta cells and many more cell types on their biologically relevant laminin is now possible. Quality and reliability is increased with recombinant laminins.

CELL CULTURE OF NEURAL CELL LINEAGES

Cell culture of dopaminergic neurons, neural stem cells, motor neurons and other nerve cells on their respective laminins increase adhesion, neurite outgrowth, maturity and functionality. Read about recommended laminins in the science room.

CLONAL PLURIPOTENT STEM CELL CULTURE

LN-521 allows efficient cell culture of single hES and iPS cells in individual wells. Use it to increase survival of single-cells at clonal densities for genome editing and single-cell sorting where survival of individual cells are needed.

CULTURE BEATING CARDIOMYOCYTES

Chemically defined generation of human cardiomyocytes is improved with cardiac laminins that increase the number and adhesion of cardiomyocytes. Isolate, expand and differentiate primary cardiac progenitors and hPSCs with muscle laminins.

MAINTAINING ENDOTHELIAL CELLS IN CULTURE

Endothelial cells and progenitors in culture can be maintained on endothelial-specific laminins for months with strong expression of endothelial marker vWF compared to fibronectin that leads to de-differentiation of endothelial cells within days.

PANCREATIC CELLS AND ISLETS

Expansion of primary pancreatic islets on pancreas-specific laminins results in proliferation of beta cells, delta cells, alpha cells and endothelial cells. Laminin-411 increase differentiation to insulin producing beta cells from mesenchymal stem cells.

RPE AND PHOTORECEPTOR CELL CULTURE

Culture retinal pigmented epithelial (RPE) cells, photoreceptors and other retinal cells on the laminins expressed in Bruch´s membrane and the neuroepithelium. Laminin-521, laminin-511, laminin-332 and laminin-111 improve RPE cell culture.

SKIN CELL CULTURE AND KERATINOCYTES

Laminin-332 is enriched in epithelial basement membranes and influences proliferation of keratinocytes and is, thus, upregulated during wound healing and importantly affects keratinocyte migration, invasion and eventually skin tissue remodeling.

HUMAN PLURIPOTENT STEM CELL CULTURE

Derivation, reprogramming, expansion and differentiation of human embryonic stem cells and induced pluripotent stem cells in completely chemically defined, feeder-free and xeno-free conditions is robust on Laminin-521 expressed by hES and iPS cells.

Read more on each cell type and which laminin to use for your cell culture in the application section of the Science Room.

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