



BIOLAMINA DEVELOPS, MANUFACTURES AND DISTRIBUTES HUMAN RECOMBINANT LAMININ CELL CULTURE REAGENTS THAT MAKES IT POSSIBLE TO CULTURE PRIMARY CELLS AND CELL LINES IN A CELL SPECIFIC AND BIOLOGICALLY RELEVANT ENVIRONMENT. LAMININ 521 IS A KEY PROTEIN OF THE NATURAL STEM CELL NICHE AND ARE EXPRESSED IN THE DEVELOPING EMBRYO AND SECRETED BY HUMAN PLURIPOTENT STEM CELLS (hPSCs) IN CULTURE. THE DEFINED AND ANIMAL COMPONENT-FREE LAMININ 521 MATRICES, BIOLAMININ 521 LN, MX AND CTG, SUPPORTS FAST CELL EXPANSION AND IS THE RECOMMENDED MATRIX FOR CULTURE OF hPSCs. BIOLAMININ 521 CREATES A MORE AUTHENTIC CULTURE ENVIRONMENT AND MAKES HANDLING OF hPSCs RELIABLE AND STANDARDIZED. FURTHERMORE, EASY AND CONTROLLED SINGLE-CELL PASSAGING CAN BE PERFORMED, TOTALLY INDEPENDENT OF ROCK INHIBITOR (ROCKi). THE CELLS GROW IN A HOMOGENEOUS MONOLAYER WITHOUT NEED FOR MANUAL REMOVAL OF DIFFERENTIATED CELL AREAS.

TRANSITION PROTOCOL

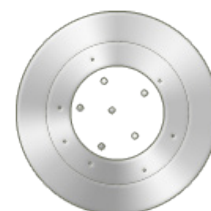
This is a protocol for the transition of hPSCs to the Biolaminin 521 cell culture matrices (LN, MX and CTG) from another feeder-free matrix (e.g. Matrigel) or from feeders.

1. Coat new cultureware with Biolaminin 521 as described in **INSTRUCTIONS FOR USE 001**.
2. For the transition step, split you cells as you normally do and then seed the cells on the Biolaminin 521 coated plate. So, if you are culturing your cells as colonies, transition to the Biolaminin 521 substrates as colonies.
 - *It is important that the cells transferred to the Biolaminin 521 matrix are of high quality. Carefully select only undifferentiated cell areas for transfer.*
 - *It is to be expected that cell morphology will look different on Biolaminin 521 compared to cells cultured as colonies on feeder or on other feeder-free matrices. The cells flatten out on the Biolaminin 521 substrate and will look bigger compared to colony cultured cells. Cell morphology may also differ depending on the medium used.*
 - *It is not recommended to change both the medium and matrix brand at the same time. Preferably, transition to the Biolaminin 521 matrix before undertaking a gradual medium transition.*
3. For the next passage, follow our protocol described in the passaging and culture instructions below. Some hPSC lines are more difficult to transition to the Biolaminin 521 matrix and might require an adaptation period (up to 5 passages) before they can be cultured as single-cells or small aggregates. To insure a successful transition, we recommend that you do the following:
 - add ROCK inhibitor (10 uM) for the first few passages
 - use a higher coating concentration (10 ug/ml)
 - seed at a higher cell density (50,000-100,000 cells/cm²) for the first few passages

Once the cells are adapted to the Biolaminin 521 matrix, the seeding density and coating concentration usually can be lowered and the hPSCs can routinely be cultured as single cells without need of ROCK inhibitor.

IMPORTANT NOTES

- All procedures should be done under sterile conditions using aseptic techniques
- The protocols can easily be made totally defined and animal component-free with your choice of culture medium and dissociation reagent
- It is important that the cells transferred to the Biolaminin 521 matrix are of high quality
- Some hPSC lines transferred to the Biolaminin 521 matrix, might require an adaptation period before they can be cultured according to the single-cell passaging protocol
- Once adapted to the Biolaminin 521 matrix, hPSCs can routinely be cultured as single cells without ROCKi
- Biolaminin 521 facilitates long-term self-renewal of hPSC without weekend feeding. For reduced labor and cost, follow the reduced feed protocol described in **APPLICATION NOTE 001**



PASSAGING PROTOCOL

The following protocol is for expansion and easy single cell passage of hPSCs on the Biolaminin 521 matrices (LN, MX and CTG). This is a generic guideline that might require optimization for best results. Volumes given in this section are for 6-well plates and should be adjusted accordingly for different sized cultureware. Once successfully transitioned and adapted to the Biolaminin 521 matrix, hPSCs can be cultured as single cells without the addition of ROCKi. Cells cultured on the Biolaminin 521 matrix are ready to be passaged when cell culture is 60-90% confluent. Note that none of the other Biolaminin matrices (except for 511) support single-cell hPSC culture. For single-cell culture of hPSCs on another Biolaminin matrix than 521 or 511, ROCKi must be added. Alternatively, mix in one part of 521 with the other Biolaminin isoform (1:3 mix) and use for coating.

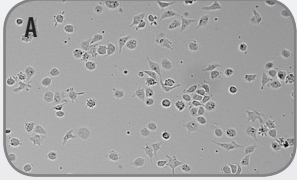
BEFORE START:

- Coat new cultureware with the Biolaminin 521 cell culture matrix as described in **INSTRUCTIONS FOR USE 001**.
 - Aliquot sufficient amounts of all solutions needed for the protocol and pre-warm to +37°C.
1. Carefully remove the excess Biolaminin coating solution from a new plate without disturbing the coated surface. Immediately add 2 mL fresh culture medium of choice to each well. Let the plate equilibrate at +37°C, with 5% CO₂ and 95% humidity.
 - Do not allow the coated surface to dehydrate as that will inactivate the Biolaminin coating.
 - Culture medium for different cell types and applications can be determined accordingly by the user. Biolaminin 521 work well in combination with most commercial media brands (e.g. NutriStem™, mTeSR™1, TeSR™2, Essential 8™ and iPS-Brew). It is to be expected that cell morphology will look different dependent on the medium used for culture.
 2. Aspirate the medium from the cells and rinse gently with 1xDPBS (Ca²⁺/Mg²⁺) (1 mL/well).
 - DPBS without Ca²⁺ and Mg²⁺ should be used since divalent cations have negative effect on some dissociating enzymes.
 - Cells cultured on the Biolaminin 521 matrix can be grown to near 100% confluence but should not grow over-confluent. Too confluent cultures will be difficult to detach.
 3. Add 1 mL/well of dissociation reagent of choice (e.g. TrypLE™, EDTA, Accutase, Trypsin) and incubate at +37°C for 3-6 minutes.
 - The incubation time is cell line and dissociation reagent dependent. Stem cells are sensitive and too much mechanical stress caused by extensive pipetting may result in low cell viability. A more confluent culture and the use of a high coating concentration could make the cells attach more tightly to the surface and a prolonged treatment time with the dissociation agent may be required. If it's still difficult to dissociate the cells, try lowering the coating concentration.
 4. Gently aspirate the cell dissociation solution and add 1-2 mL/well of pre-warmed fresh medium. Gently pipette up and down 2-4 times to achieve single-cell suspension or small aggregates. The mechanical force applied should be minimal not to cause significant physical damage to the cells.
 - Use a microscope to verify that the cells are properly dissociated. When using Biolaminin 521, we recommend passaging pluripotent stem cells as single cells or as small aggregates. When using EDTA, try to achieve as small cell aggregates as possible without using too much mechanical force. Extensive pipetting may result in low cell viability. Rather, increase the incubation time to minimize pipetting.
 5. Collect the cell suspension in a 15 mL conical tube. Centrifuge at 100 x g for 4 minutes at room temperature (+15°C to +25°C). If you are using EDTA as a dissociation agent, you can skip step 5 and 6.
 6. Aspirate and discard the supernatant and gently resuspend the cell pellet in fresh, pre-warmed culture medium of choice. For each well of hPSCs collected, add 1-2 mL of medium.
 7. Count the cells and calculate the cell suspension volume needed for seeding. hPSC should be seeded with a density of 30,000-50,000 cells/cm² or with a split ratio of 1:10 to 1:30.
 - Optimal seeding densities will vary from one cell line to another. The Biolaminin 521 culture system is flexible and the split ratio can be adjusted empirically for each protocol and need. Biolaminin 521 can support cell survival at a seeding density as low as 5,000 cells/cm².
 - When transitioning your cells to Biolaminin 521 from another feeder-free matrix (e.g. Matrigel) or from feeder cells, follow the instructions in the **TRANSFER PROTOCOL** above.
 8. Bring out the fresh Biolaminin 521 coated tissue culture plate containing fresh, pre-warmed culture medium and transfer the desired volume of cell suspension to each well. Immediately rock the plate to get an even cell distribution.
 - If you are transitioning your cells to Biolaminin 521 from feeders or another feeder-free matrix, we recommend adding ROCK inhibitor to a final concentration of 10 μM for the first few passages. Once adapted to the Biolaminin matrix, pluripotent stem cells can routinely be cultured as single cells without the need for ROCK inhibitor.
 9. Place the plate into the incubator and culture the cells at +37°C, with 5% CO₂ and 95% humidity. Perform a complete medium change 24-48 hours after passaging and then feed cells on daily basis until the next passage. For reduced labor and cost, follow the weekend-free protocol described in APPLICATION NOTE 001.
 - Within 60 minutes, the majority of the cells should have attached, evenly distributed as single cells across the well. The day after seeding the cells should have formed small colonies. hPSCs cultured on Biolaminin 521 should grow as a homogenous monolayer, without any differentiated areas. See representative pictures below.

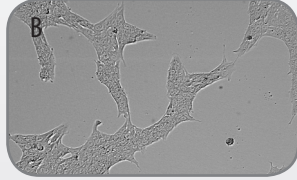


MORPHOLOGY OF hESC AT VARIOUS DAYS AFTER SEEDING

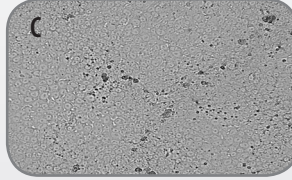
Representative pictures (10x magnification) of human embryonic stem cell (hESC) line HS181 seeded as single cells (30,000 cell/cm²) in Nutristem™ medium onto 5 µg/mL of Biolaminin 521 (pictures A-C), or seeded as aggregates in mTeSR™ medium on Matrigel according to the manufacturer's instructions (picture D). Within 1 hour after seeding on LN521, the majority of the cells should have attached, evenly distributed across the surface (A). The cells show high motility and will migrate to make contact with other cells, initiating proliferation. The day after seeding, the majority of the cells should have formed small colonies (B). Cells seeded on LN521 should grow as a homogenous monolayer. The cells should exhibit a cobblestone morphology with high nuclear-to-cytoplasm ratio and prominent nucleoli (C). Unlike colony passaging on other feeder-free matrices (D), cells cultured as single-cells on Biolaminin 521 can be cultured to near confluence without signs of spontaneous differentiation (C). It is to be expected that cell morphology will differ between single cell (C) and colony (D) cultured cells. Cell morphology may also differ depending on the medium used for culture.



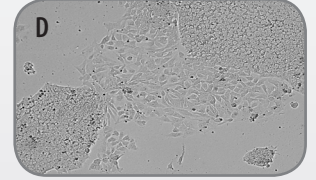
Day 0: hESC HS181 cultured on LN521, 1 hour after seeding. The cells have attached and are evenly distributed as single cells.



Day 1: hESC HS181 cultured on LN521, the day after seeding. The cells have formed small colonies.



Day 4: hESC HS181 cultured on LN521, just before passage. Confluent cell monolayer without differentiated cell areas.



hESC HS181 cultured as colonies on Matrigel, 3 days after seeding. Area of differentiation between 2 undifferentiated colonies.

THAWING CRYOPRESERVED hPSCs

Thawed hPSCs should be seeded into Biolaminin 521 coated wells. hPSCs cultured on another feeder-free matrix or feeders can be thawed directly onto Biolaminin 521. At the first passage, follow the single-cell **PASSAGING PROTOCOL**. When the cell transition to the Biolaminin 521 matrix is problematic, follow instruction in the **TRANSFER PROTOCOL** above.

BEFORE START:

- Coat new cultureware with Biolaminin 521 as described in **INSTRUCTIONS FOR USE 001**. If unsure of the number of cells / aggregates frozen down, a 6-well plate is recommended.
 - Aliquot sufficient amounts of all solutions needed for the protocol and pre-warm to +37°C.
1. Carefully remove the Biolaminin coating solution from a new plate and immediately add 2 mL fresh culture medium of choice to each well. Let equilibrate at +37°C, with 5% CO₂ and 95% humidity.
 - The coating does not require washing before use.
 - Do not allow the coated surface to dehydrate.
 - Culture medium can be determined accordingly by the user.
 2. Quickly thaw the hPSCs in a +37°C water bath by gently shaking the cryovial continuously until only a small frozen pellet remains.
 3. Sterilize the cryovial with 70% ethanol and carefully transfer the cell suspension to a 15 mL conical tube.
 4. Gently add 5-7 mL of pre-warmed medium of choice. Centrifuge at 100 x g for 4 minutes at room temperature (+15°C to +25°C).
 5. Discard the supernatant and gently resuspend the cell pellet in 1-2 mL of fresh, pre-warmed culture medium.
 6. Count the cell number (if applicable) and transfer the appropriate amount of cells suspension to a Biolaminin 521 coated well plate.
 - About 1 million cells/well in a total volume of 2 mL is recommended if using a 6-well plate (adjusted accordingly for other well size).
 7. Place the plate into the incubator and gently rock the plate to distribute the cells evenly.
 8. Culture the cells at +37°C, with 5% CO₂ and 95% humidity. Perform a medium change after 24-48 hours and then on daily basis.

CRYOPRESERVING hPSCs

hPSCs cultured on Biolaminin 521 should be cryopreserved as single cells or small aggregates when 60-70% confluent. The cryopreservation medium should be defined, serum-free and designed specifically for hPSCs. Volumes given in this section are for 6-well plates and should be adjusted accordingly for different sized cultureware.

BEFORE START:

- Aliquot sufficient amounts of all solutions needed for the protocol and pre-warm to +37°C. The cryopreservation medium should be handled according to the manufacturer's Instructions.
 - Prepare and label cryovials.
1. Aspirate the medium from the cells and rinse gently with 1 mL/well of 1xDPBS (Ca²⁺/Mg²⁺).
 - DPBS *without* Ca²⁺ and Mg²⁺ should be used since divalent cations have negative effect on some dissociating enzymes.
 2. Add 1 mL/well of dissociation reagent of choice (e.g. TrypLE™, EDTA, Accutase, Trypsin) and incubate at +37°C for 3-6 minutes.
 - The incubation time is cell line and dissociation reagent dependant. It also depends on the coating laminin concentration used and the degree of cell confluence.
 3. Gently aspirate the dissociation solution and add 1-2 mL/well of pre-warmed fresh medium. Gently pipette up and down 2-4 times to achieve single-cell suspension or small aggregates.
 - When using EDTA, try to achieve as small cell aggregates as possible without using too much mechanical force.
 4. Collect the cell suspension in a 15 mL conical tube. Centrifuge at 100 x g for 4 minutes at room temperature (+15°C to +25°C).
 5. Aspirate and discard the supernatant. Gently resuspend the cell pellet in 2 mL cryopreservation medium of choice. Count the cells (if applicable) and transfer 0.5-1 mL of cell suspension (0.5 - 1x10⁶ cells/mL) into cryovials.
 6. Freeze cells using a standard slow rate controlled protocol (approx. -1°C/min) and store at -135°C to -180°C.

BIOLOGICALLY RELEVANT CELL CULTURE SUBSTRATES FOR DIFFERENT CELL APPLICATIONS

Most organized cells in the human body grow on a basement membrane that contains laminins, an interaction that is essential for their survival and tissue specific functions. With BioLamina's human, recombinant laminin cell culture matrices, Biolaminins, it is now possible to culture pluripotent stem cells, adult stem cells and tissue-specific cells in a cell specific and physiologically relevant environment. Our Biolaminin

matrices are defined and consistent, making cell culture easy, standardized and more authentic. The Biolaminin cell culture matrices have shown to improve the expansion and maturation of many cell types, such as pancreatic beta cells, cardiomyocytes, different kind of neurons, neural stem cells, endothelial cells, cancer stem cells and many more. Below are a few examples.



HUMAN PLURIPOTENT STEM CELL CULTURE

Robust derivation, reprogramming, expansion and differentiation of human ES and iPS cells under completely chemically defined, feeder-free and animal component-free conditions on Biolaminin 521. Biolaminin 521 also increase survival and expansion of single cells at clonal densities.



RPE AND PHOTORECEPTOR CELL CULTURE

Culture retinal pigmented epithelial (RPE) cells, photoreceptors and other retinal cells on laminin isoforms 521, 511, 332 and 111, expressed in Bruch's membrane and the neuroepithelium. Biolaminin 521 support efficient production of functional hESC-RPE cells.



HEPATOCTE DIFFERENTIATION AND MATURATION

Biolaminin 521 and 111 support efficient specification and maturation of hESC-derived hepatocytes with significantly increased metabolic activity and functional organization.



PANCREATIC B-CELLS ISLETS

Efficient maintenance and expansion of primary pancreatic islets on pancreas-specific Biolaminin 521 and 421. Biolaminin 411 increase differentiation of mesenchymal stem cells into insulin producing beta cells.



CULTURE BEATING CARDIOMYOCYTES

Chemically defined isolation, expansion and differentiation of primary cardiac progenitors and hPSCs-derived cardiomyocytes is improved with cardiac laminins 521, 221 and 211.



SKIN AND HAIR CELL CULTURE

Laminin 332 and 511 is enriched in epithelial basement membranes and influences proliferation and migration of keratinocytes during wound healing. Laminin 332 and 511 is the major laminin of the hair follicle.



MAINTAINING ENDOTHELIAL CELLS IN CULTURE

Efficient differentiation of hESC to endothelial progenitor cells using Biolaminin 521. Human endothelial progenitor cells can also efficiently be maintained on endothelial-specific laminin.



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.

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

www.thescienceroom.com

✉ KEEP IN TOUCH

TEL: +46-8-5888 5180

FAX: +46-8-5198 9288

EMAIL: INFO@BIOLAMINA.COM



BIOLAMINA AB

LÖFSTRÖMS ALLÉ 5A

172 66 SUNDBYBERG (STOCKHOLM)

SWEDEN

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