BioLamina
REVOLUTIONIZING CELL CULTURE

# CULTURING HUMAN PSCs
## ON HUMAN RECOMBINANT LAMININ-521

INSTRUCTIONS FOR USE 003

BIOLAMINA DEVELOPS, MANUFACTURES AND DISTRIBUTES LAMININ (LN) CELL CULTURE REAGENTS THAT MAKES IT POSSIBLE TO CULTURE PRIMARY CELLS AND CELL LINES IN A CELL SPECIFIC AND BIOLOGICALLY RELEVANT ENVIRONMENT. ALPHA-5 LAMININS (LN-521 AND LN-511) ARE KEY PROTEINS OF THE NATURAL STEM CELL NICHE AND ARE EXPRESSED IN THE DEVELOPING EMBRYO AND SECRETED BY HUMAN PLURIPOTENT STEM CELLS (hPSCs) IN CULTURE. LN-521 SUPPORTS FASTER CELL EXPANSION COMPARED TO LN-511 AND IS THE RECOMMENDED MATRIX FOR CULTURE OF hPSCs. THE DEFINED AND XENO-FREE LN-521 CELL CULTURE MATRIX CREATES A MORE AUTHENTIC CULTURE ENVIRONMENT THAT SUPPORTS ROBUST CELL EXPANSION 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.

## TRANSFER PROTOCOL

The transfer of hPSCs from another feeder-free matrix (e.g. Matrigel) or from feeders to the LN-521 cell culture matrix is often quite straight forward. Coat a new plate with the LN-521 matrix according to **INSTRUCTIONS FOR USE 001** and perform single-cell passage as described in the **PASSAGING PROTOCOL** below. It is to be expected that cell/colony morphology will look different when compared to cells grown using other culture conditions.

- *It is important that the cells transferred to the LN-521 matrix are of high quality. Carefully select only undifferentiated cell areas for transfer. Initially, it's recommended to seed 2-3 wells of a smaller well format (e.g. 48-well format), for the option to choose a well with most homogeneous cell population for further use.*
- *Use a higher seeding density (50,000-100,000 cells/cm$^2$) for the first number of passages before lowering the seeding density.*
- *It is not recommended to change both medium and matrix brand at the same time. Preferably transfer the hPSCs to the LN-521 matrix before doing the medium transition the next day.*

## IMPORTANT NOTES

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

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Some hPSC lines are more difficult to transfer and might require an adaptation period before they can be cultured as single cell as described in the **PASSAGING PROTOCOL** described below. When the cell transfer to the LN-521 matrix is problematic, try the following:

1. Increase the coating concentration to 10 ug/mL. Once the cells are adapted, a lower coating concentration can often be used which should be optimized empirically for each cell line.
2. Transfer and culture the cells as small aggregates or as single cells in combination with ROCKi for a few passages. Once adapted, hPSCs can routinely be cultured as single cells without ROCKi, as described below. It may take up to 5 passages for some cell lines become acclimatized to LN-521.

# PASSAGING PROTOCOL

The following protocol is for easy single cell passage of hPSCs on LN-521. 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 transferred and adapted to the LN-521 matrix, hPSCs can be cultured as single cells without the addition of ROCKi. Cells cultured on the LN-521 matrix are ready to be passaged when cell culture is 60-99% confluent. Depending on the cell line, seeding density and on the medium used, cultures are usually passaged 3-6 days after seeding. Note that none of the other laminin matrices (except for LN-511) support single-cell hPSC culture. For single-cell culture of hPSCs on another laminin matrix than LN-521 or LN-511, ROCKi must be added. Alternatively, mix in one part of LN-521 with the other laminin matrix for support (1:3 mix) and use for coating.

BEFORE START:

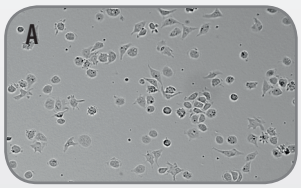
- Coat new cultureware with the LN-521 cell culture matrix as described in **INSTRUCTIONS FOR USE 001** or use 521-To-Go™ pre-coated plates as described in **INSTRUCTIONS FOR USE 007.**
- Aliquot sufficient amounts of all solutions needed for the protocol and pre-warm to +37°C.

1. Carefully remove the excess laminin coating solution from a new plate without disturbing the coated surface. Immediately add 2 mL fresh culture medium of choice to each well. Let equilibrate at +37°C, with 5% $CO_2$ and 95% humidity.
   - *Do not allow the coated surface to dehydrate as that will inactivate the laminin coating.*
   - *Culture medium for different cell types and applications can be determined accordingly by the user. LN-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 ($Ca^{--}$/$Mg^{--}$) (1 mL/well).
   - *DPBS without $Ca^{2+}$ and $Mg^{2+}$ should be used since divalent cations have negative effect on some dissociating enzymes.*
   - *Cells cultured on the LN-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™ or EDTA) and incubate at +37°C for 3-6 minutes.
   - *The incubation time is cell line and dissociation reagent dependent. It also depends on the laminin coating concentration used and the degree of cell confluence. More confluent cultures might need longer treatment time compared to sub-confluent cultures. However, stem cells are sensitive and too long exposure to enzymes or too much mechanical stress caused by extensive pipetting may result in low cell viability. If it's still difficult to dissociate the cells, try lowering the laminin coating concentration.*
4. Gently aspirate the dissociation solution and add 1-2 mL/well of pre-warmed fresh medium. Gently pipette up and down 4 - 8 times to achieve single-cell suspension. 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. It's harder to get the cells into a single cell suspension using EDTA compared to enzymatic dissociation. 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).
6. Aspirate and discard the supernatant and gently flick the tube to fully dislodge the cell pellet from the tube bottom. 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 cell number and plate the cells onto the LN-521 coated plate prepared in step 1. hPSC should be seeded with a density of 30,000-50,000 cells/$cm^2$ or with a split ratio of 1:10 to 1:30.
   - *Optimal seeding densities will vary from one cell line to another.*
   - *The LN-521 culture system is flexible and the split ratio can be adjusted empirically for each protocol and need. LN-521 can support cell survival at a seeding density as low as 5,000 cells/$cm^2$.*
   - *When transferring your cells from another feeder-free matrix (e.g. Matrigel) or from feeder cells, follow the instructions in the* **TRANSFER PROTOCOL** *above.*
8. Place the plate into the incubator and gently rock the plate to distribute the cells evenly.
9. Culture the cells at +37°C, with 5% $CO_2$ and 95% humidity. 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.
   - *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 LN-521 should grow as a homogenous monolayer, without any differentiated areas. The cells should exhibit a high nuclear- to-cytoplasm ratio and prominent nucleoli.*
   - *With optimal media conditions and seeding density, most cell lines will reach confluence within 4-6 days and expand 10-25 fold.*
   - *For reduced labor and cost, follow the weekend-free protocol described in* **APPLICATION NOTE 001**.

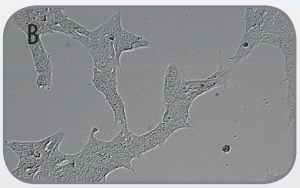
# MORPHOLOGY OF hESC AT VARIOUS DAYS AFTER SEEDING

Representative pictures (10x magnification) of human embryonic stem cell (hESC) line HS181 seeded as single cells (30,000 cell/cm$^2$) in Nutristem™ medium onto 5 ug/mL of LN-521 (pictures A-C), or seeded as aggregates in mTeSR™ medium on Matrigel according to the manufacturers instructions (picture D). Single cells seeded on LN-521 should grow as a homogenous monolayer, without any differentiated areas. It is to be expected that cell morphology will look different dependent on the medium used for culture.

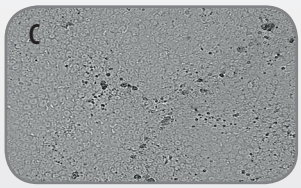
Within 1 hour after seeding, the majority of the cells should have attached to the LN-521, evenly distributed across the surface (A). The cells show high motility on LN-521 and will migrate to make contact with other cells, initiating proliferation. The day after seeding, the majority of the cells should have formed small colonies (B). The cells should exhibit a high nuclear-to-cytoplasm ratio and prominent nucleoli. The cells are ready to be passaged when cell culture is 60-99% confluent (C). Unlike colony passaging on other feeder-free matrices (D), cells cultured as single-cells on LN-521 can be cultured to near confluence without signs of spontaneous differentiation (C).



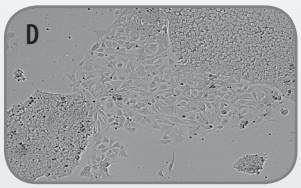

**Day 0**: hESC HS181 cultured on LN-521, 1 hour after seeding. The cells have attached and are evenly distributed as single cells.




**Day 1**: hESC HS181 cultured on LN-521, the day after seeding. The cells have formed small colonies.




**Day 4**: hESC HS181 cultured on LN-521, 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 LN-521 coated wells. hPSCs cultured on another feeder-free matrix or feeders can be thawed directly onto LN-521, as single cells or as aggregates. At the first passage, follow the single-cell **PASSAGING PROTOCOL**. When the cell transfer to the LN-521 matrix is problematic, follow instruction in the **TRANSFER PROTOCOL** above.

## BEFORE START:

- Coat new cultureware with LN-521 as described in **INSTRUCTIONS FOR USE 001** or use 521-To-Go™ pre-coated plates as described in **INSTRUCTIONS FOR USE 007.** 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 laminin 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% $CO_2$ and 95% humidity.
   - *The laminin coating does not require washing before use.*
   - *Do not allow the coated surface to dehydrate.*
   - *Culture medium for different cell types and applications 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 LN-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% $CO_2$ and 95% humidity. Feed cells on daily basis.

# CRYOPRESERVING hPSCs

hPSCs cultured on LN-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 ($Ca^{--}$/$Mg^{--}$).
   - *DPBS without $Ca^{2+}$ and $Mg^{2+}$ 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™ or EDTA) 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.*
   - *Stem cells are sensitive and too long exposure to enzymes or too much mechanical stress may result in low cell viability.*
3. Gently aspirate the dissociation solution and add 1-2 mL/well of pre-warmed fresh medium. Gently pipette up and down 4 - 8 times to achieve single-cell suspension.
   - *It's harder to get the cells into a single cell suspension using EDTA compared to enzymatic dissociation. 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 -1x10$^6$ 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.

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# BIOLOGICALLY RELEVANT LAMININ FOR CELL SPECIFIC APPLICATIONS

**Most cells in the human body grow on specific laminins, an interaction that is essential for their survival and tissue specific functions. With BioLamina's human, recombinant laminin (LN) cell culture matrices, 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 laminin matrices are defined and consistent, making cell culture easy, standardized and more authentic. The laminin cell culture matrices have shown to improves 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.**

## HUMAN PLURIPOTENT STEM CELL CULTURE

Robust derivation, reprogramming, expansion and differentiation of human ES and iPS cells under completely chemically defined, feeder-free and xeno-free conditions on LN-521. LN-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 the laminin isoforms LN-521, LN-511, LN-332 and LN-111, expressed in Bruch´s membrane and the neuroepithelium. LN-521 support efficient production of functional hESC-RPE cells.

## HEPATOCYTE DIFFERENTIATON AND MATURATION

LN-521 and LN-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 LN-521 and LN-421. LN-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 LN-521, LN-221 and LN-211.

## SKIN AND HAIR CELL CULTURE

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

## MAINTAINING ENDOTHELIAL CELLS IN CULTURE

Efficient differentiation of hESC to endothelial progenitor cells using LN-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.

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**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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