S04E

Laminins and cell culture

Recreating the natural cell niche is key to successful cultivation. The physical, topological, and biochemical expression of the different laminin isoforms in the BMatrice is heterogeneous and tissue specific. Here is a superior set of Laminins to design the best cell culture conditions for your cell line.

Technical data - laminins

Product number	LN521	LN511	LN421	LN411	LN211	LN121	LN111
Protein name	Laminin 521	Laminin-511	Laminin-421	Laminin-411	Laminin-211	Laminin-121	Laminin-111
Synonyms	Laminin-11	Laminin-10	Laminin-9	Laminin-8	Laminin-4	Laminin-3	Laminin-1
Product application search by Cell Lines search by Applicationss	Human embryonic and induced pluripotent stem cells	Murine embryonic and induced pluripotent stem cells	Endothelial cells, mesangial cells and maturation of neuromuscular junctions	Pancreatic islet culture, endothelial cells, adipocytes and epidermal cells	Skeletal and smooth muscle cells, cardiomyocytes, glia cells and neural progenitor cells	General cell culture substrate	General cell culture substrate
Use concentration *	0.5-2 µg/cm^2	0.5-2 µg/cm^2	0.5-2 µg/cm^2	0.5-2 µg/cm^2	0.5-2 µg/cm^2	0.5-2 µg/cm^2	0.5-2 µg/cm^2
Expressed in vivo	Expressed and secreted by human pluripotent stem cells and can, in addition, also be found in the kidneys, neuromuscular junctions, lungs and placenta.	Expressed and secreted by human pluripotent stem cells and can, in addition, also be found in epithelia and dermal papillae, endothelial cells, pancreas, peripheral nerves, and placenta.	Expressed and secreted by mesangial cells, endothelial cells and can, in addition, also be found in the presynaptic terminal of neuromuscular junctions.	Expressed and secreted by endothelial cells, adipocytes, lymphocytes and platelets, and muscle cells as well as pancreatic cells, salivary and gastric glands, and epidermal cells in the developing and adult human body.	Expressed and secreted by skeletal muscle cells and cardiomyocytes.	Expressed and secreted by trophectoderm and some epithelial basement membranes. Can thus be found in the placenta, skeletal muscles and kidneys.	Expressed and secreted by trophectoderm and some epithelial basement membranes

*Optimal concentration must be determined by the end user

All laminins store at -20°C (I), stability is 3 years from date of receipt

Set of 8 Laminins (LAMscreen)

Laminin Cell Culture Applications - Biorelevant cell culture conditions

HUMAN PLURIPOTENT STEM CELLS & CLONAL CELL APPLICATIONS
Embryonic stem cells: LN521
Induced pluripotent stem cells: LN521
Pluripotent stem cell clonal culture: LN521

RETINAL CELLS, CORNEAL CELLS & PHOTORECEPTOR CELLS
Retinal pigmented epithelial cells: LN521, LN511, LN332
Human corneal endothelial cells: LN521, LN511
Photoreceptors: LN332, LN532, LN432

THYMOCYTES & IMMUNE CELLS
Thymocytes & T cells: LN411, LN332, LN21

SKELETAL MUSCLE CELLS
Myogenic stem cell progenitors: LN521
Skeletal mucle cells: LN211, LN521, LN221, LN511
Smooth muscle cells: LN211, LN521, LN221, LN511

HEPATOCYTES & LIVER CELLS
Hepatocytes: LN521, LN411, LN111, LN221
Cholangiocytes: LN521, LN411, LN111, LN221

PANCREATIC ISLETS & BETA CELLS
Insulin producing cells: LN411, LN521, LN511, LN421
Primary pancreatic islet cells: LN521, LN511, LN411

ADIPOSE CELLS
Adipocytes: LN411

HAIR FOLLICLE CELLS & SKIN CELLS
Hair follicle stem cells: LN511, LN332
Keratinocytes: LN521, LN511, LN332
Intestinal epithelial cell: LN511, LN521

NEURONS, GLIA CELLS & NEURAL STEM CELLS
Neural stem cells: LN521, LN511, LN111
Dopaminergic neurons- LN111, LN521
Motor neurons: LN211
Cortical neurons: LN521, LN411, LN211
Sensory neurons: LN511, LN411, LN111
Interneurons: LN521, LN411, LN421, LN211
Astrocytes: LN521, LN211, LN111
Schwann cells: LN211, LN411
Oligodendrocytes: LN21

MAMMARY GLAND CELLS
Mammary epithelial cells: LN521, LN511, LN111

LUNG CELLS
Lung: LN521, LN511, LN411, LN111

CARDIOMYOCYTES
Cardiomyocytes: LN521, LN211, LN511, LN221

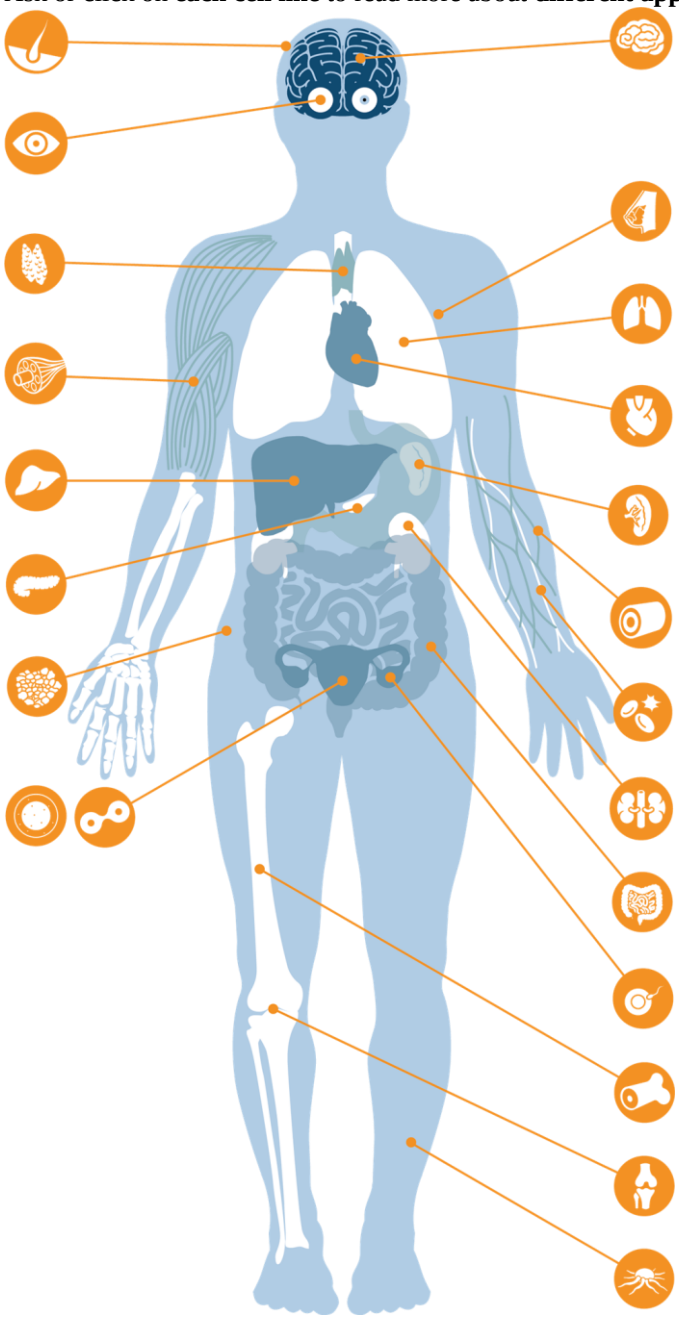
SPLEEN CELLS
Spleen: LN521

ENDOTHELIAL CELLS
Endothelial cells: LN521, LN411, LN511

HEMATOPOIETIC STEM CELLS & BLOOD CELLS
HSC: LN521, LN511, LN421, LN411
Megacaryocytes: LN521

We offer an complete portfolio of chemically defined and xeno-free laminin proteins for cell culture matrices in a variety of applications, to imitate the natural, cell-specific cell-matrix interaction *in vitro*, including reliable expansion of pluripotent cell and differentiation and maintenance of specialized cell types.

Ask or click on **each cell line** to read more about **different applications**



HEMATOPOIETIC STEM CELLS & BLOOD CELLS
HSC: LN521, LN511, LN421, LN411
Megacaryocytes: LN521

KIDNEY CELLS
Glomerular endothelial cells: LN521, LN411, LN111
Tubular epithelial cells: LN521, LN511
Podocytes: LN521, LN511
Mesangial cells: LN421

INTESTINAL CELLS
Intestine: LN511, LN521, LN111

GERM CELLS
Oocytes: LN521
Testis: LN211, LN22

MESENCHYMAL STEM CELLS
MSC: LN521, LN511, LN421, LN411

BONE & CARTILAGE CELLS
Osteoblasts: LN521
Chondrcytes: LN521, LN421

MESENCHYMAL STEM CELLS
MSC: LN521, LN511, LN421, LN411

CANCER CELLS
Glioblastoma: LN521
Breast cancer stem cells: LN511

ANIMAL STEM CELLS

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e-mail interchim@interchim.com - web www.interchim.com

Publications – laminin 511

•Laminin-511 but not -332, -111, or -411 enables mouse embryonic stem cell self-renewal in vitro.
Domogatskaya A, Rodin S, Boutaud A, Tryggvason K.
Stem Cells, 2008

•Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511.
Rodin S, domogatskaya A, Ström S, Hansson EM, Chien KR, Inzunza J, Hovatta O, Tryggvason K.
Nature Biotechnology, 2010

•Xeno-free culture of human pluripotent stem cells.
Bergström R, Ström S, Holm F, Feki A, Hovatta O.
Methods in Molecular Biology, 2011

•Compositional and structural requirements for laminin and basement membranes during mouse embryo implantation and gastrulation.
Miner JH, Li C, Mudd JL, Go G, Sutherland AE.
Development, 2004

•Trophoblastspecific expression and function of the integrin alpha 7 subunit in the peri-implantation mouse embryo.
Klaffky E,Williams R,Yao CC, Ziober B, Kramer R, Sutherland AE.
Developmental Biology, 2001

LAMscreen Laminins set

LAMscreen kit contains all currently available laminin isoforms in smaller quantities (20 ug of 8 laminins amongst the 16 known)) which provides an easy and efficient way to test which laminins that are optimal for your culturingfor your cells.

Size: 140µg 8 x 20 ug
Stock concentration: 0.1mg/ml
Use concentration: 0.5-2 ug/cm2
Contains:
Laminin-521, laminin-511, laminin-421, laminin-411, laminin-221, laminin-121, laminin-211 and laminin-111
Synonyms: Laminin-11, laminin-10, laminin-9, laminin-8, laminin-4, laminin-3, laminin-2 and laminin-1

Laminins are expressed tissue-specifically in basement membranes of the extracellular matrix lining all epithelia-endothelia and covering other cell/organ types such as adipose tissue and muscle fibers.

FREEZEstem™ DMSO-Free Cryopreservation Of Cells PC521 1R5450

Laminin 521 STEM CELL MATRIX (LN-521)

Laminin-521 (LN-521) is a key cell adhesion protein of the natural stem cell niche, expressed and secreted by hPSCs in the inner cell mass of the embryo. LN-521 recreates the biologically relevant milieu in vitro and therefore supports robust expansion of human ES and iPS cells.

The LN-521 stem cell substrate enables high expansion rate, monolayer homogenous growth, and maintained pluripotency. Cells are genetically stable without karyotypic abnormalities, showed with both karyotyping and SNP arrays.

hESC and iPSC grow faster on LN-521 compared to other feeder-free matrices, facilitating automation. Once adapted to the LN-521 matrix, hPSCs can routinely be cultured as single cells without the addition of ROCKi. LN-521 also supports efficient clonal survival and expansion of hES and iPS cells, greatly facilitating the study of genomic alterations and manipulations in pluripotent cells. Furthermore, renders more efficient reprogramming of specific tissue cells to iPSCs.

The robust support of pluripotent stem cells by laminin-521 enables maintenance of hPSC without weekend feeding.

After birth, LN-521 also represent one of the most common laminins in the body and together with the other laminin isoforms, it supports expansion and differentiation of many diverse tissue cell types.

KEY BENEFITS OF LN-521 FOR hPSC CULTURE:

- **Defined and xeno-free** cell culture matrix: for control and clinical compliance
- **No lot-to-lot variability**: more reliable and standardized experiments
- Homogenous hPSC cultures that are pluripotency and genetically stabile: no need to remove differentiated cell areas
- **High clonal survival**: optimal for derivation and gene editing
- **Triggers authentic cell signalling pathways**: provides hPSCs cultures with a more uniform gene expression profile
- **High expansion rate and controlled single-cell passaging**: facilitates automation and high-throughput processes
- Authentic milieu that primes the hPSCs: more efficient differentiation and enhanced cell maturation, polarization and organization
- **Flexible culture system**: easy to control and adaptable to any protocol
- Support weekend feeding: no more weekend work!

Laminin-521 (LN-521) is the natural laminin for pluripotent stem cells and therefore reliably facilitates self-renewal of human ES and iPS cells in a chemically defined, feeder-free and xeno-free stem cell culture system. LN-521 enables efficient single-cell passaging of genetically stable and pluripotent stem cells without need of any apoptosis inhibitors for superior quality of your cells and studies.
After birth, α5-laminins represent the most common laminins in the body and LN-521 supports many diverse tissue cell types, such as cells from pancreas, vascular, nervous and muscular systems.

Laminin 521, Human, recombinant

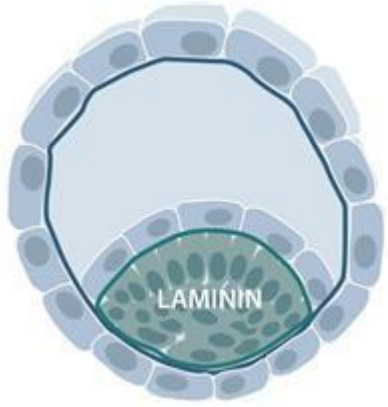
LN-521-01, 100µg LN-521-02, 1mg LN-521-03, 10x1mg

521-To-Go™ LN-521 precoated plates

PC521-024-01, 24 well , 1 plate PC521-024-05, 24 well, 5 plates PC521-024-20, 24 well, 20 plates

Supporting data – laminin 521

Biological relevance



Laminin-521 (LN-521) is a key cell adhesion protein of the natural stem cell niche, expressed and secreted by hPSCs in the inner cell mass of the embryo. LN-521 recreates the biologically relevant milieu in vitro and therefore supports robust expansion of human ES and iPS cells.

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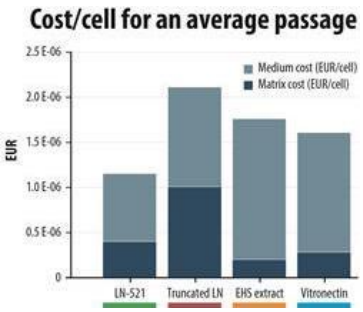
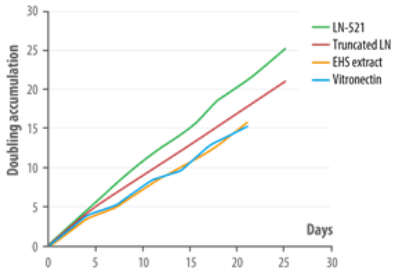
Cost-effective stem cell culturing



The specific LN-521 binding of the α6β1 integrin enables high cell survival and self-renewal and hPSCs grow faster on LN-521 compared to other feeder-free matrices. Due to faster growth rate and higher cell yield, the total cost (medium and matrix) per cell for an average passage is lowest for LN-521.

Long-term efficient expansion



hESC and iPSC grow faster on LN-521 compared to other feeder-free matrices, facilitating automation. The specific LN-521 binding of the α6β1 integrin enables high cell survival and self-renewal. Cells expand quickly and reach 100% confluency after only 4 days after a 1:10 split. You will thus have 10.000 fold more cells after only 4 passages, making the cell quantities of low-passage hPSCs needed for clinical applications possible.

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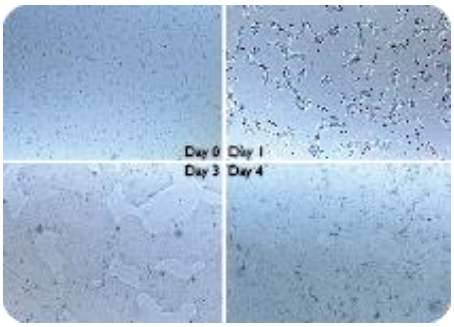
Genetic stability

With LN-521 hPSCs can be seeded as single cells at low densities and cultured long-term without introduction of genetic abnormalities. Cells have been kept on LN-521 for more than 130 passages with stable karyotypes. Importantly, when LN-521 is used for the derivation of new hESC lines, the embryo is not destroyed and the lines originating from a single blastomer are genetically stable.

Homogenous hPSC monolayer



PSCs plated as single-cell suspension on LN-521 grow as a homogenous monolayer without any abnormal genetic aberrations. The specific LN-521 binding of the α6β1 integrin enables high cell survival and self-renewal and once adapted to the LN-521 matrix, hPSCs can routinely be cultured as single cells without the addition of ROCKi. LN-521 also support clonal survival and is a good substrate for derivation and gene editing.

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Less labor and complexity

hPSCs can be cultured to near confluence on LN-521 without spontaneous differentiation. The cells remain pluripotent (Oct4+; pink) on LN-521 with no sopntaneous differentiation and there is no need to remove differentiated cell areas (only DAPI; blue) as compared to cells cultured on other substrates.

Easy and flexible culture protocols

The single-cell split is easy, fast and forgiving, making anyone suitable to culture hPSCs. When using LN-521, your cell culture protocol can easily be made totally defined and xeno-free with your choice of culture medium and dissociation reagent. For reduced labor and cost, the LN-521 matrix supports weekend-free feeding.

- Coat plates with LN-521.
- Wash the cells with PBS and add dissociation reagent of choice - incubate
- Centrifuge the single-cell suspension and resuspend the pellet in fresh medium of choice
- **Seed as single-cell suspension on fresh LN-521 coated plates**

Protocols – laminin 521

COATING PLATES

See Technical sheet

521LAh CULTURING HUMAN PSCs On Human Recombinant Laminin-521 [0]

JV0360 COATING PLATES WITH HUMAN RECOMBINANT LAMININS FOR CELL CULTURE

hPSC CULTURE ON LN-521

See Technical sheet

521LAh CULTURING HUMAN PSCs On Human Recombinant Laminin-521, including:

- transfer
- passaging
- thawing
- cryopreservation of hPSCs on LN-521.

521-To-Go™ pre-coated plates

1R5450 Instruction fo 521-to-Go Laminin521 coated plates [0]

Embryoid body formation

See the technical sheet

521LAe EMBRYOID BODY FORMATION from hPSCs Cultured On LN521 [0]

Applications notes:

JV036a TISSUE SPECIFIC LAMININ CELL CULTURE MATRICES applications poster [0]

521LAw WEEKEND-FREE CULTURE OF HUMAN PLURIPOTENT STEM CELLS ON LN-521 (appl.note001)

521LAp LN-521 STEM CELL MATRIX derivation and expansion of pluripotent cells [0]

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
- 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.

Troubleshooting – laminin 521

Laminin plate coating

An uneven cell spread, it's often a coating issue and could be caused by the following:

- To low coating concentration used. Increase the coating concentration is high enough to support an even cell growth.
- DPBS Ca--/Mg-- has been used. We recommend to use DPBS with Ca^{2+} and Mg^{2+} since divalent cations are important for the protein structure and function.
- NUNC-plates was used. Most plastic work well for laminin coating but we know the laminin coating is not compatible with some NUNC-plates. SARSDET and Corning plate usually work well.
- Bad coating coverage/the plate has dried out. Ensure that the entire surface is covered by the laminin coating solution when preparing fresh plates. Also, do not let the plate dry out as this will inactivate the laminin coating. Too long time in the incubator or long storage without sealing could cause too much evaporation so that part of the plate dries out (often center).

Transfer from other substrates

- We recommend to transfer the cells as single cells (or as small clumps) and always with the addition of ROCKi for the first few (3-5) passages. Once the cells are adapted to the LN-521 matrix, the cells can be cultured as single cells without ROCKi. This may take up to 5 passages.
- If the cells are hard to adapt, try increasing the coating concentration to 10 ug/ml and seed at a higher cell density 50,000 – 100,1000 cells/cm^2. Once the cells are adapted a lower coating and seeding concentration often can be used.
- It is important that the cells transferred to the LN-521 matrix are of high quality. LN-521 will generally also support differentiated cells so carefully select only undifferentiated cell areas for transfer.
- LN-521 work well in combination with most commercial media brands. However, it is to be expected that cell morphology will look different dependent on the medium used for culture.

hPSC splitting and seeding

- We recommend single-cell passage or passage as small clumps.
- Stem cells are sensitive and when using enzyme, do not treat the cells too long as that will damage the cells. Cells attach tighter on laminin compared to other matrices and scraping or pipetting without first loose up cells can affect cell integrity and viability which could result in less attachment next day. Less confluent cells need shorter treatment time whereas more confluent cells might need longer treatment time. The cells should detach easily without too much pipetting needed. Do not use too much mechanical force (extensive pipetting or scraping) as that will damage the cells. Increase the dissociation reagent incubation time rather than increasing the force. If the cells stick very hard to the LN-521 surface, try to lower the coating concentration.
- After seeding: most cells should have attached after 1 hour and the cells should be evenly distributed over the entire plate. If there is a lot of cell death after seeding, the cells have most likely been treated too harsh during splitting.
- The day after seeding: the cell has migrated and should have formed small colonies and will continue to expand as a homogenous monolayer. 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.

Publications – Laminin 521

•Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment.

Sergey Rodin, et *al* . Nature Communications, 2014

This article provides scientific evidence that LN521™ is the optimal matrix for generation and culture of human pluripotent stem cells. It is described in detail how this physiologically relevant laminin establishes genetically stable hESC lines in an efficient, defined, xeno-free and feeder-free procedure, suitable for stem cell banking and regenerative medicine applications.

•A defined xeno-free and feeder-free culture system for the derivation, expansion and direct differentiation of transgene-free patient-specific induced pluripotent stem cells.

Hong Fang Lu, Chou Chai, Tze Chiun Lim, Meng Fatt Leong, Jia Kai Lim, Shujun Gao, Kah Leong Lim, Andrew C.A. Wan. Biomaterials, 2014

This article demonstrates LN521 as an optimal defined, xeno- and feeder-free matrix for the reprogramming of human iPS cells. LN521 achieves high-efficiency reprogramming in different media (E8, Nutristem, etc), fast and easy expansion as well as direct differentiation to dopaminergic neurons on LN521. The authors conclude that the efficient transgene-free hiPSC derivation and expansion on LN521 enables clinical applications useful for human patient iPSCs and derivatives for cellular therapy.

•Optimization of slow cooling cryopreservation for human pluripotent stem cells.

Takamichi Miyazaki, Norio Nakatsuji and Hirofumi Suemori. Genesis, 2013

This is one of the first customer publications that demonstrates LN521 as an optimal xeno- and feeder-free matrix for pluripotent stem cells. The authors show cells should be cryopreserved as single cells for highest survival which is specifically supported by LN521 that promotes adhesion and self-renewal of fully dissociated single cells in the absence of ROCK inhibitor. They demonstrate 80-90% survival of hPSCs post-thawing and 60% survival following subculture on LN521, allowing for efficient and easy handling of cells and bulk storage of high-quality hPSCs.

•Live visualization of chromatin dynamics with fluorescent TALENs.

Yusuke Miyanari, Céline Ziegler-Birling & Maria-Elena Torres-Padilla. Nature structural & molecular biology, 2013

The authors of this study used LN511 as a coating substrate for mouse embryonic stem cells on glass in order to aquire better images for the study of endogenous repetitive genomic sequences visualized by TALE integration.

•Efficient and Scalable Expansion of Human Pluripotent Stem Cells Under Clinically Compliant Settings: A View in 2013.

Ying Wang, Linzhao Cheng and Sharon Gerecht. Annals of Biomedical Engineering, 2013

The authors of this important review highlight the capacity of LN521 and LN511 to successfully support long-term expansion of pluripotent stem cells.

•Xeno-free culture of human pluripotent stem cells.

Bergström R, Ström S, Holm F, Feki A, Hovatta O. Methods Mol Biol, 2011

A book chapter describing four different animal-protein free culture systems that are proven efficient in expanding pluripotent human ES cell populations. Two of the systems are with LN511 as substrate but with different medium, and the other two substrates are CELLstart and human foreskin fibroblasts. Control of ground-state pluripotency by allelic regulation of Nanog Miyanari Y, Torres-Padilla ME Nature, 2012 The authors of this study used LN511 as a coating substrate for mouse embryonic stem cells to investigate the role of Nanog for ground-state pluripotency during development. By using LN511 they were able to culture the mouse embryonic stem cells as monolayers and importantly without the use of LIF in a completely defined cell culture environment.

LN511 Human recombinant Laminin-511

Human recombinant laminin-511 (LN-511) is the natural laminin for mouse embryonic stem cells with sustained pluripotency without the need to use feeder cells or differentiation inhibitors like LIF.

Laminin-511 is together with laminin-521 (LN-521) the most widely expressed laminins in the body. LN-511 is especially recommended for cell culture of murine embryonic stem cells or induced pluripotent stem cells while LN-521 is recommended for human pluripotent stem cells.

Culture of mouse pluripotent stem cells on LN-511, which is already expressed at the 4-cell stage during embryonic development, maintains the pluripotency without the use of LIF. Using LN-511 for adherent murine stem cell cultures makes culturing easy and efficient.

Laminin 511, Human, recombinant

LN-521-01, 100µg LN-521-02, 1mg LN-521-03, 10x1mg

Applications

Mouse ES and iPS cells grow on laminin-511 (LN-511) in a completely defined matrix, without the need of using time-consuming and cumbersome feeder cells or LIF since the laminin-511 protein creates a biological niche keeping the ES cells in a pluripotent state for months. BioLamina's LN-511 is a full-length human recombinant laminin that is used as a mouse ESC or iPSC culture substrate.

Stem cells grow as monolayers on LN-511 enabling equal contact to both the matrix and cell culture medium, which leads to a more homogenous cell population. Since LN-511 is a recombinant protein, lot-to-lot variation is minimal and therefore fewer replicates are needed for reliable results (Domogatskaya, 2008; Rodin, 2010).

The need for matrices that support pluripotent stem cell renewal in a reproducible manner is clear. Stem cell biologists have long experienced problems with the repeatability of results and with maintenance of pluripotency when working with ES cell cultures.

CULTURE OF MURINE STEM CELLS ON LAMININ-511

Additives like the cytokine LIF have thus been added to the cell culture medium to minimize spontaneous cell differentiation or one has needed to culture the cells on feeder cell layers.

Now, however, LN-511 allows the proliferation of pluripotent mouse ES cells without such differentiation inhibitors or feeder cells. Importantly, the ES cells grow as a homogenous monolayer, which ensures equal availability to both substrate and soluble factors and, thus, creates a more homogenous cell population (Domogatskaya, 2008).

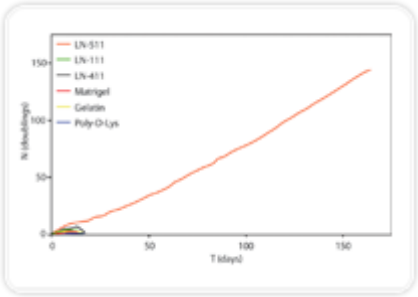
LN-511 and other laminins can also be applied to glass, and as stem cells grow as a monolayer on LN-511 and LN-521 these laminins have been used for improved imaging of stem cells grown on glass slides.

The LN-511 matrix provides better opportunities for designing experiments and interpreting obtained results.

LN-511 can also be used for culture of many other cell types as seen under laminin applications.

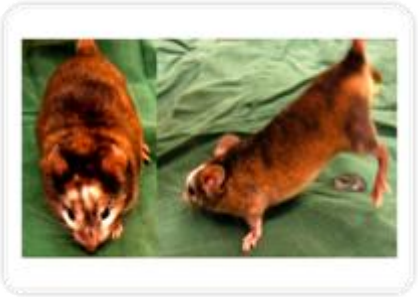
Supporting data – laminin 521

mESC expansion without LIF



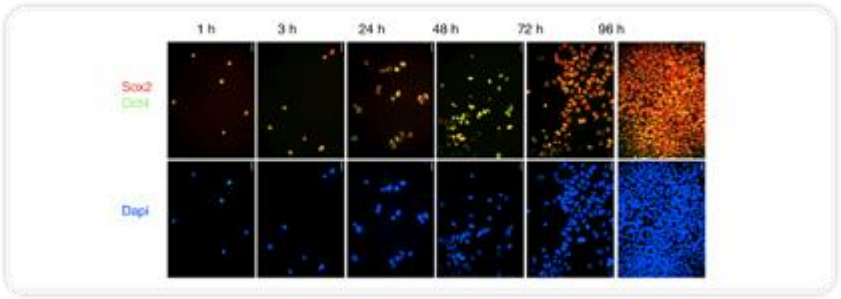
LN-511 support the self-renewal of mouse ES cells for over 5 months without the presence of LIF or feeder cells, when other known matrices are unable to do so for longer than a couple of weeks.

.Long-term pluripotent mouse ESC



Mouse ES cells grown on LN-511 for over 3 months are indeed pluripotent since they give rise to germline transmission (chimeric mice) after injection into mouse blastocysts.

Homogenous monolayer



Mouse ES cells adhere to LN-511 with three to five-fold higher affinity than to other commonly used matrices. Pluripotent mouse ES cells grow even at low cell density as monolayers on the surface of LN-511, which is different from the conventional clusters in the cultures, and, therefore, have equal contact with the matrix and medium, allowing a homogeneous and defined growth environment.

Protocols – Laminin 511

PASSAGE MOUSE PSC ON LAMININ-511

1. Aspirate old medium from wells.

2. Wash the cells gently with 1xDPBS (Ca++/Mg++) and aspirate, add TrypLE to wells and incubate at room temperature for max 30 seconds.

3. Aspirate TrypLE, and wash the cells twice with pre-warmed medium.

4. Add fresh medium to wells. Scrape cells with a pipette tip, then gently pipette up and down to achieve a clump size of approximately 0.5 -1 mm in diameter. Avoid making single-cell suspension.

5. Seed cells with a ratio of 1:2, 1:3 or 1:4 on LN511 coated wells. Optimal seeding densities will vary from one cell line to another and can be determined empirically for your system.

6. Swing plates side-to-side to distribute cell aggregates evenly, then place them into an incubator (+37°C, 5% CO2).

See details in technical sheet 511LA0 - Laminin511-Culturing ES and iPS Cell on human Laminin [0]

COATING PROCEDURE

1. Slowly thaw recombinant laminins at +4°C before use. 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 and diluted coating solution can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C.

2. Dilute the thawed laminin stock solution with 1xDPBS containing Ca2+ and Mg2+.

3. Add the diluted laminin solution to tissue culture-treated cultureware for a final coating concentration of 0.5-2 ug/cm2. The optimal coating concentration is cell line-dependent.

4. Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours. Make sure the laminin solution is spreaded evenly across the surface. Note that the laminin matrix will be inactivated if let dry.

See more details in the Technical sheet JV0360

IMPORTANT NOTES

•The laminin stock solution is stable for 2 years when stored at -20°C. If desired, the laminin stock can be dispensed into working aliquots and stored at -20°C. Repeated freeze thawing should be avoided.

•When culturing mouse PSCs on LN-511 no treatment with differentiation inhibitors, such as leukemia inhibitory factor (LIF), is needed.

•The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.

Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C, 5% CO2.

•Cells are ready to be passaged when cell culture is ≥60% confluent. Optimal seeding densities will vary from one cell line to another and can be determined empirically for your system. With optimal media conditions and seeding density, most cell lines will reach confluence within 4-6 days and expand 10-25 fold.

•When moving your cells from another feeder-free matrix (e.g. Matrigel) we recommend you to start smaller well format (e.g. 96-well or 48-well format) and a higher seeding density (50 000-100 000 cells/cm2) 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.

See protocol in the technical sheet Laminin511-Culturing ES and iPS Cell on human Laminin

LN421 Human recombinant Laminin-421

Human recombinant laminin-421 supports several tissue-specific cell types such as endothelial cells and kidney cells, and has been suggested to be important for renal, synaptic and inflammatory functions.

Applications

In the kidney, laminin-421 is produced by mesangial cells and is presumably important for the development and reparation of microvasculature and glomerulogenesis (Abrass, 2010) whereas kidney podocytes mainly bind to laminin-521 via the α3β1 integrin (Sachs and Sonnenberg, 2013). Laminin-421 has also been implicated in renal cell carcinomas (Lohi, 1996; Vainionpää, 2007) and glial brain tumours (Ljubimova, 2001).

In chemical synapses, the laminin-421 isoform is crucial for the correct localization of pre- and postsynaptic specializations. In mice lacking the laminin β2 chain, neuromuscular junctions fail to form active zones in the presynaptic terminal and thereby neurotransmitter release is disrupted (Patton, 2001). Laminin-421 has been shown to assist in the formation and stabilization of active zones by several mechanisms, including voltage-gated Ca2+-channels and integrins containing the α3 subunit (Carlsson, 2003; Cohen, 2000).

Protocols – Laminin 421

COATING PROCEDURE

1. Slowly thaw recombinant laminins at +4°C before use. 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 and diluted coating solution can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C.

2. Dilute the thawed laminin stock solution with 1xDPBS containingCa2+ and Mg2+.

3. Add the diluted laminin solution to tissue culture-treated cultureware for a final coating concentration of 0.5-2 ug/cm2. The optimal coating concentration is cell-dependent.

4. Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours. Make sure the laminin solution is spreaded evenly across the surface. Note that the laminin matrix will be inactivated if let dry.

See more details in the Technical sheet JV0360

IMPORTANT NOTES

- The Laminin stock solution is stable for 2 years when stored at -20°C. If desired, the laminin stock can be dispensed into working aliquots and stored at -20°C. Repeated freeze thawing should be avoided.
- The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.
- Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C, 5% CO2.
- Coat the wells in advance with the laminin solution according to the instruction here.

LN411 Human recombinant Laminin-411

Recombinant human laminin-511 and laminin-411 can in combination create a natural niche in the culture dish for endocrine pancreatic beta cells, promoting both proliferation of β cells and insulin gene expression.

In the embryo, differentiation and insulin expression by the pancreas β cells is initiated by and dependent on specific signals from capillary endothelial cells (Lammert, 2001). However, pancreatic cells are unable to produce the extracellular proteins laminin-411 and laminin-511, which are expressed and secreted by endothelial cells.

Instead, the pancreatic islets express vascular endothelial growth factor to attract endothelial cells, providing both oxygen and nutrients and form the essential vascular network for insulin production by the β cells (Lammert, 2003; Nikolova, 2006).

Data presented by Nikolova (2006) and colleagues suggest that treating islets with these laminins prior to transplantation will help maintain insulin production until new capillaries are formed in transplanted islets and that laminin-411 and laminin-511 in cell culture potentially could mimic the biological environment in the pancreas.

BioLamina's revolutionary matrix system brings together the unique qualities of laminin-411 and laminin-511 to provide the solution for making pancreatic islet cells thrive.

Laminin 411, Human, recombinant

LN-411-01, 100µg LN-411-02, 1mg LN-411-03, 10x1mg

DIFFERENTIATION AND MAINTENANCE OF PANCREATIC CELLS

One of the main isoform of laminins in capillaries and larger vessels produced by endothelial cells is laminin-411 and, thus, it has a pivotal role in endothelial basal laminae function (Kortesmaa, 2000; Qian, 2007; Wondimu, 2004). Transgenic mice with an α4-chain laminin deficiency exhibit severe disorganization of microvessels, leading to microcirculation abnormalities (Thyboll, 2002; Wang, 2006).

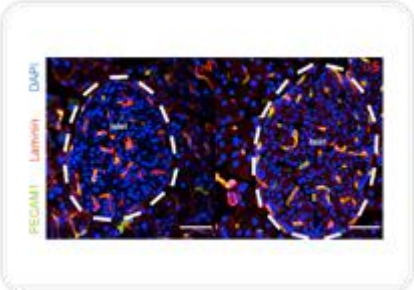
In addition, during sprouting angiogenesis laminins are produced and deposited from the stalk cells to the tip cell filopodia, and control the diameter size of the vessel lumen (Jakobsson, 2008), further supporting the importance of the protein in endothelial structure and function.

Lymphoid cells are derived from bone marrow stem cells and differentiate either in the thymus or in the bone marrow. Lymphocytes are fundamental for innate and adaptive immunity and patrol the body for foreign antigens that activates them. Laminin-411 is secreted by lymphocytes and supports the proliferation, adhesion and migration of lymphocytes (Geberhiwot, 2001).

Laminin-411 is also involved in the survival, adhesion and migration of blood neutrophils (Wondimu, 2004), and works as an adhesive substratum for bone marrow stem and progenitor cells and promote progenitor cell migration in vitro (Gu, 2003).

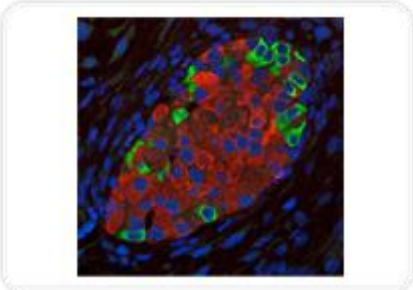
Applications

Laminins in the pancreas



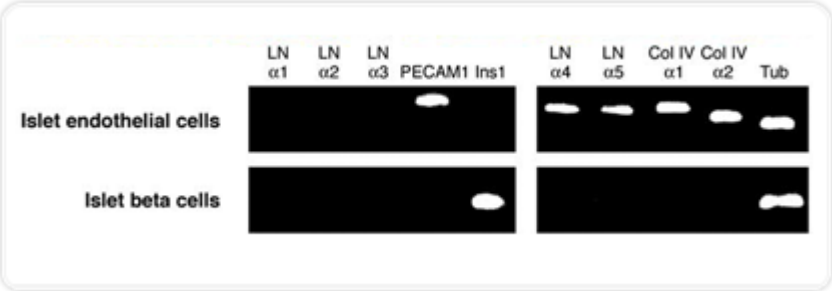
Islet endothelial cells in mouse pancreas express both laminin-411 (left) and laminin-511 (right) seen as red in these confocal microscopy images. The laminins induce a cell-signaling cascade in the β islet cells, which leads to expression of insulin and cell proliferation.

Laminins and insulin



Laminin-411 and laminin-511 in cell culture can partially substitute the biological environment of the pancreas and can, therefore, potentially help maintain β cell insulin production during islet transplantations before the islets are revascularized by the body.

Laminins by endothelial cells



Laminins and other extracellular proteins, including collagen IV, are only produced by islet endothelial cells and not by islet β cells. Laminin-411 and laminin-511 proteins act in concert with instructive and cell-type specific signals and are, in addition, required in liver morphogenesis and growth.

Protocols

COATING PROCEDURE

1. Slowly thaw recombinant laminins at +4°C before use. 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 and diluted coating solution can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C.

2. Dilute the thawed laminin stock solution with 1xDPBS containingCa2+ and Mg2+.

3. Add the diluted laminin solution to tissue culture-treated cultureware for a final coating concentration of 0.5-2 ug/cm2. The optimal coating concentration is cell-dependent. See laminin coating instructions, recommended coating volumes and concentrations.

4. Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours. Make sure the laminin solution is spreaded evenly across the surface. Note that the laminin matrix will be inactivated if let dry.

See details in the technical sheet JV0360

IMPORTANT NOTES

- The laminin stock solution is stable for 2 years when stored at -20°C. If desired, the laminin stock can be dispensed into working aliquots and stored at -20°C. Repeated freeze thawing should be avoided.
- The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.
- Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C, 5% CO2.
- Coat the wells in advance with the laminin solution according to the instruction here.

LN221 Human recombinant Laminin-211

Human recombinant LN221 supports the growth, survival and differentiation of a wide range of tissue-specific cell types, including cardiac cells and skeletal muscle cells.

Applications

Laminin-221 is important for muscle development and function and is togheter with laminin-211 one of the main lamionin isoforms present in adult muscle tissue, including varying amounts of laminin-521 and laminin-421 depending on tissue.

Mutations of the LAMA2 gene are the most common cause of congenital muscular dystrophy that frequently leads to death in early childhood (Domogatskaya, 2012). For a review on α2-laminin in skeletal muscle function, see Holmberg and Durbeej, 2012.

Laminin-211 as well as laminin-221, are very important for cardiomyocytes and heart muscle development. In the developing heart both laminin-211 and laminin-221 are expressed in the extracellular matrix of cardiomyocytes as well as in the basement membrane zones of the endo- and pericardium and the capillaries (Roediger, 2010).

Laminins containing the α2-chain (such as laminin-221) are expressed in the ventricular zone ECM in the developing mouse central nervous system. Since laminins are known to contribute to the stem cell niche in the brain, this suggests that laminin-221 could play an important functional role in the regulation of neural stem cells and progenitor cells (Haubst, 2006; Kazanis, 2010; Lathia, 2007; Mercier, 2002).

Laminin 211, Human, recombinant

LN-221-01, 100µg LN-221-02, 1mg LN-221-03, 10x1mg

Protocols

COATING PROCEDURE

1. Slowly thaw recombinant laminins at +4°C before use. 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 and diluted coating solution can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C.

2. Dilute the thawed laminin stock solution with 1xDPBS containingCa2+ and Mg2+.

3. Add the diluted laminin solution to tissue culture-treated cultureware for a final coating concentration of 0.5-2 ug/cm2. The optimal coating concentration is cell-dependent. Coat the wells in advance with the laminin solution according to the instruction here.

4. Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours. Make sure the laminin solution is spreaded evenly across the surface. Note that the laminin matrix will be inactivated if let dry.

See details in the technical sheet JV0360

IMPORTANT NOTES

- The laminin stock solution is stable for 2 years when stored at -20°C. If desired, the laminin stock can be dispensed into working aliquots and stored at -20°C. Repeated freeze thawing should be avoided.
- The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.
- Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C, 5% CO2.
- Coat the wells in advance with the laminin solution according to the instruction here.

LN121 Human recombinant Laminin-121

THIS PRODUCTS IS CURRENTLY ONLY AVAILABLE AS PROTOTYPE MATERIAL

Laminins-111/121 are widely expressed during embryogenesis, important for early epithelial development and differentiation of the epiblast thus vital for the development of an embryo However, the tissue distribution after birth is restricted to only a few tissues, such as brain and kidney.

Laminin-121 can be used as a general attachment protein for most cell types in vitro and in particular for hepatic and neural differentiation, and to enhance neurite outgrowth.

Laminin 121, Human, recombinant

LN-121-01, 100µg LN-121-02, 1mg LN-121-03, 10x1mg

Applications

Laminin-121 can support the survival, proliferation and differentiation of many different cell types in vitro, although many of those cultivated cell types would not encounter the protein naturally in vivo. Instead you should match your specific cell type to the laminin naturally expressed by this cell which leads to improved cell culture.

In the adult, laminin α1 chain expression is relatively restricted and is only present in some epithelial basement membranes, and primarily found in tissues such as the eye, liver and kidney, whereas hardly expressed at all in endothelial, adipose, nervous, and muscle tissues (Ekblom, 2003; Virtanen, 2000).

Commonly, laminin-111 have been used as a general attachment protein for most cell types in vitro. Laminin-121 has not been commercially available until now and therefore the functional comparison between LN121, LN111 and other laminins isoforms is yet to be performed in order to understand the potential for LN121 for cells in culture.

However, known is that the laminin beta 2 chain (LN121) has been suggested to have a higher affinity for integrins compared to the alfa 1 chain (LN111) thus influencing stability of intergin-laminin and activation of downstream effectors in cell signaling cascades.

It is unknown why laminin-121 can exert all these major biological effects on such a wide selection of cell types, but one explanation is that, depending on the cell type, laminin-121 can not only induce different cell signaling cascades but as all laminins also co-signal with various growth factors.

However, by coating your plate with recombinant laminin-121 you support your cells with a defined and xeno-free substrate. Therefore, human recombinant laminin-121 enables better control of your cell matrix and experiments.

Protocols

COATING PROCEDURE

1. Slowly thaw recombinant laminins at +4°C before use. 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 and diluted coating solution can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C.

2. Dilute the thawed laminin stock solution with 1xDPBS containingCa2+ and Mg2+.

3. Add the diluted Laminin solution to tissue culture-treated cultureware for a final coating concentration of 0.5-2 ug/cm2. The optimal coating concentration is cell-dependent. See laminin coating instructions, recommended coating volumes and concentrations.

4. Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours. Make sure the laminin solution is spreaded evenly across the surface. Note that the laminin matrix will be inactivated if let dry.

See details in the technical sheet JV0360

IMPORTANT NOTES

- The Laminin stock solution is stable for 2 years when stored at -20°C. If desired, the laminin stock can be dispensed into working aliquots and stored at -20°C. Repeated freeze thawing should be avoided.
- The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.
- Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C, 5% CO2.
- Coat the wells in advance with the laminin solution according to the instruction here.

LN111 Human recombinant Laminin-111

Human recombinant laminin-111 is important during early epithelial development and differentiation of the epiblast. Laminin-111 is up-regulated and widely expressed during embryogenesis, vital for the development of an embryo. However, its tissue distribution after birth is restricted to only a few tissues, such as brain and kidney. Laminin-111 is commonly used as a general attachment protein for most cell types in vitro and LN111 have been used successfully for hepatic and neural differentiation.

Laminin 111, Human, recombinant

LN-111-01, 100µg LN-111-02, 1mg LN-111-03, 10x1mg

Applications

Laminin-111 supports the survival, proliferation and differentiation of many different cell types in vitro, although many of those cultivated cell types would not encounter the protein naturally in vivo. Instead you should match your specific cell type to the laminin naturally expressed by this cell which leads to improved cell culture.

During development Riechert's membrane supporting the extraembryonic trophoblasts express laminin-111. Reichert's membrane is essential for epiblast differentation, which is essential for the formation of the three embryonic germ layers by pluripotent cells in the inner cell mass.

In the adult, laminin α1 chain expression is relatively restricted and is only present in some epithelial basement membranes, and primarily found in tissues such as the eye, liver and kidney, whereas hardly expressed at all in endothelial, adipose, nervous, and muscle tissues (Ekblom, 2003; Virtanen, 2000).

It is unknown why laminin-111 can exert all these major biological effects on such a wide selection of cell types, but one explanation is that, depending on the cell type, laminin-111 can not only induce different cell signaling cascades but as all laminins also co-signal with various growth factors.

Laminin-111 is the laminin isoform present in the tumor extract sold under the trade name Matrigel. Matrigel is produced by mouse Engelbreth-Holm-Swarm (EHS) sarcoma cells and is an undefined mix of extracellular proteins of animal tumor origin.

However, by coating your plate with recombinant laminin-111 you support your cells with a defined and xeno-free substrate. Therefore, human recombinant laminin-111 enables better control of your cell matrix and experiments.

Laminin-111 is the main component in Matrigel that is used for stem cell culture. By using matrigel for human stem cells, laminin-111 initiates differentiation of stem cells instead of efficient self-renewal of stem cells. This can be seen as spontaneous differentiation of pluripotent stem cells in Matrigel or laminin-111 coated cell culture dishes.

For pluripotent stem cells, laminin-521 should instead be used for human stem cell culture and laminin-511 for mouse stem cell culture. This is because laminin-521 and laminin-511 are the natural laminins for hES and iPS cells and does not cause spontaneous differentiation. Instead they lead to efficient expansion and growth of pluripotent stem cells.

Protocols

COATING PROCEDURE

1. Slowly thaw recombinant laminins at +4°C before use. 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 and diluted coating solution can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C.

2. Dilute the thawed laminin stock solution with 1xDPBS containingCa2+ and Mg2+.

3. Add the diluted Laminin solution to tissue culture-treated cultureware for a final coating concentration of 0.5-2 ug/cm2. The optimal coating concentration is cell-dependent. See laminin coating instructions, recommended coating volumes and concentrations.

4. Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours. Make sure the laminin solution is spreaded evenly across the surface. Note that the laminin matrix will be inactivated if let dry.

See details in the technical sheet JV0360

IMPORTANT NOTES

- The Laminin stock solution is stable for 2 years when stored at -20°C. If desired, the laminin stock can be dispensed into working aliquots and stored at -20°C. Repeated freeze thawing should be avoided.
- The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.
- Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C, 5% CO2.
- Coat the wells in advance with the laminin solution according to the instruction here.

Related documents

***** technical sheets and application notes:

JV0360 COATING PLATES WITH HUMAN RECOMBINANT LAMININS FOR CELL CULTURE

JV036a TISSUE SPECIFIC LAMININ CELL CULTURE MATRICES applications poster [0]

521LAh CULTURING HUMAN PSCs On Human Recombinant Laminin-521 [0]

521LAe EMBRYOID BODY FORMATION from hPSCs Cultured On LN521 [0]

521LAw WEEKEND-FREE CULTURE OF HUMAN PLURIPOTENT STEM CELLS ON LN-521 (appl.note001)

521LAp LN-521 STEM CELL MATRIX derivation and expansion of pluripotent cells [0]

1R5450 Instruction fo 521-to-Go Laminin521 coated plates [0]

511LA0 Laminin511-Culturing ES and iPS Cell on human Laminin [0]

511LAa LN-511 LIF INDEPENDENT MURINE STEM CELL EXPANSION [0]

411LAp LN-511 and LN-411 MAINTENANCE OF PANCREATIC CELLS IN VITRO [0]

Associated products

1R5450 FREEZEstem™ DMSO-Free Cryopreservation Of Cells #PC521

Related products/documents

Products HighLights Overview

Information inquire

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