

Application Note 001

Proliferation of pluripotent human ES and iPS cells in a defined, xeno-free environment

Laminin-511

Culturing human embryonic stem (ES) cells in a controlled and repeatable manner is known to be difficult. Researchers have tried for years to find a solution to this crucial problem. BioLamina now provides Laminin-511, a natural hES cell niche that solves this problem.

Recombinant human Laminin-511 is a protein matrix that for the first time creates a defined environment for infinite and pluripotent growth of hES and iPS cells. Together with an appropriate cell culture medium, this offers several key advantages:

1. Proliferation of pluripotent hES and iPS cells for months in cell culture
2. Repeatability between experiments – cells grow as monolayers in a 100%-defined environment
3. Biological relevance – Laminin-511 is the natural niche for hES cells, binding the four-cell stage embryo together
4. Fewer replicates to get trustworthy results
5. Direct transfer of methods to production of clinical-grade material since Laminin-511 can be manufactured according to GMP

Proliferation of pluripotent human ES and iPS cells in a defined, xeno-free environment

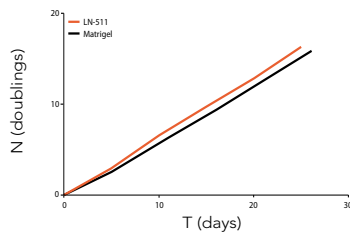


FIGURE 1: Human embryonic stem cells can be cultured continuously in a pluripotent state on recombinant Laminin-511 for over 5 months. Data shows cells cultured for 5 months.



FIGURE 2: Pluripotent hES and iPS cells grow as monolayers on top of Laminin-511 for over 5 months. All cells have equal contact to the matrix and medium, allowing a homogeneous growth and defined environment.

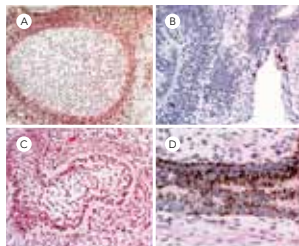


FIGURE 3: Stem cells grown on Laminin-511 over 3 months can produce teratomas of all three germ layers. A, Cartilage. B, Developing neural tissue. C, Developing kidney glomerulus. D, Retinal pigment epithelium.

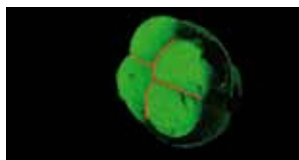


FIGURE 4: Laminin-511 is the first extracellular protein expressed by the developing embryo, already at the 4-cell stage. As a bio-relevant glue it binds the developing cells in the embryo together. Laminin is illustrated in red.

REFERENCES

Rodin S, Domogatskaya A, Ström S, Hansson EM, Chien KR, Inzunza J, Hovatta O, Tryggvason K.: Long-term self-renewal of human embryonic stem cells on human recombinant laminin-511 in xeno-free and feeder-free environment. *Nature Biotechnology*. May 30 2010 doi:10.1038/nbt.1620.

Ordering information

PRODUCT	SUITABLE CELL TYPES	APPLICATIONS	QUALITY	PROD NO.	QUANTITY
Human Recombinant Laminin-511	Mouse and human ES iPS cells Other multipotent cells	Expansion of pluripotent ES, iPS cells and other multipotent cells	Non-GMP	50511 50512 50513	100 µg 10 µg 1000 µg

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Application Note 002

Self-renewal of mouse ES cells without LIF

Laminin-511

The need for matrices that support pluripotent embryonic stem (ES) cell renewal in a reproducible manner is clear. Stem cell biologists have long experienced problems with proliferating ES cells in the pluripotent form and with repeatability of the results.

Chemical additives like leukemia inhibitory factor (LIF) have thus been added to the cell culture medium to prevent cells differentiating. Now, however, Laminin-511 allows proliferation of mouse ES cells in a pluripotent form without any such artificial differentiation inhibitors. This protein is the only known matrix that provides a defined, xeno-free and biorelevant cell culture environment. Furthermore, cells grow as a homogenous monolayer, which ensures uniform results.

Laminin-511, the first extracellular protein expressed already in the four-cell stage embryo, is a biorelevant glue that binds embryonic cells together. This function is most likely the reason why Laminin-511 supports pluripotent mouse ES cell proliferation so well, even in the complete absence of differentiation inhibitors. It is also the most probable explanation for why mouse ES cells adhere to Laminin-511 with about five-fold higher affinity than other tested matrices, allowing cells to grow as monolayers.

The Laminin-511 matrix thus provides several key advantages:

1. Lack of mES cell differentiation eliminates the need for LIF
2. High repeatability between experiments – cells grow as monolayers in a 100%-defined environment
3. Proliferation of pluripotent mES cells for several months in cell culture
4. Fewer replicates needed to get trustworthy results
5. Biological relevance – ES cells grow on Laminin-511 in the embryo

Self-renewal of mouse ES cells without LIF

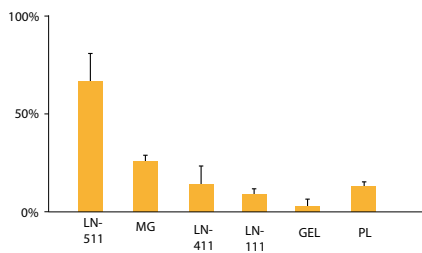


FIGURE 1: mES cells adhere to Laminin-511 with about three to five-fold higher affinity than to other commonly-used matrices. Values are shown as average percentage of cells that attached.



FIGURE 2: Pluripotent mES cells grow pluripotently as monolayers on the surface of Laminin-511 for over 5 months. All cells have equal contact with the matrix and medium, allowing a homogeneous and defined growth environment.



FIGURE 3: Stem cells grown on Laminin-511 for over 3 months give a germline transmission when injected into blastocysts.

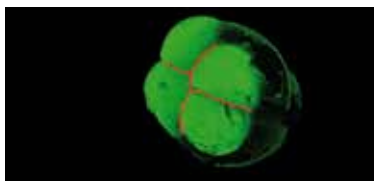


FIGURE 4: Laminin-511 is the first extracellular protein expressed by the developing embryo, already at the four-cell stage. As a bio-specific glue, it binds the developing cells in the embryo together. Laminin is illustrated in red.

REFERENCES

Domogatskaya A, Rodin S, Boutaud A, Tryggvason K.:Laminin-511 but not -332, -111, or -411 enables mouse embryonic stem cell self-renewal in vitro. Stem Cells 26, 2800-2809, 2008.

Ordering information

PRODUCT	SUITABLE CELL TYPES	APPLICATIONS	QUALITY	PROD NO.	QUANTITY
Human Recombinant Laminin-511	Mouse and human ES iPS cells Other multipotent cells	Expansion of pluripotent ES, iPS cells and other multipotent cells	Non-GMP	50511 50512 50513	100 µg 10 µg 1000 µg

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Application Note 003

Creating a niche for insulin-producing pancreatic β cells in vitro

Laminin-511, Laminin-411

Culturing pancreatic β cells that produce insulin in cell culture is difficult. In the embryo, differentiation of insulin-producing β cells from pancreatic epithelium cells strictly requires endothelial cells. Later, delaminated β cells aggregate to form islets.

These islets are dependent on extracellular signaling for insulin production, but do not themselves express extracellular proteins. Instead, the pancreatic islets attract endothelial cells that express extracellular proteins such as Laminin-511 and Laminin-411, both of which are crucial for the production of insulin by β cells.

Results published by Nikolova *et al* in *Developmental Cell* suggest that:

1. Laminin-411 and Laminin-511 in cell culture can partially substitute the biological environment of the pancreas
2. Laminin-411 and Laminin-511 can help maintain β cell insulin production during islet transplants before the islets are revascularized by the body
3. The Laminin-411 and Laminin-511 proteins act in concert with instructive and celltype specific signals and can be required in liver morphogenesis and growth

Creating a niche for insulin-producing pancreatic β cells in vitro

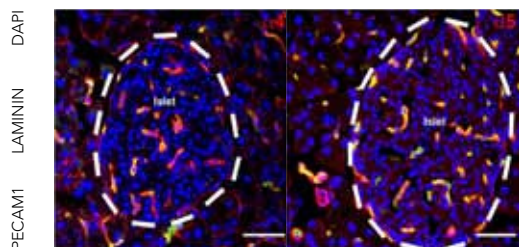


FIGURE 1: Both Laminin-411 and Laminin-511 are expressed by endothelial cells and are present in the islets (red). The laminins cause a cell-signaling cascade in the β islet cells, which leads to expression of insulin. Laminin-411 (left) and Laminin-511 (right) are shown in red in these confocal microscopy images.

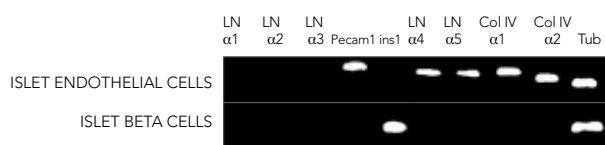


FIGURE 2: Laminin (LN) and other extracellular proteins including Collagen IV are only produced by the islet endothelial cells and not by the islet β cells. PECAM used as a positive control for islet endothelial cells and insulin-1 (Ins1) for islet β cells, show no cross-contamination between the sorted cell populations.

REFERENCES

Nikolova, G., Jabs, N., Konstantinova, I., Domogatskaya, A., Tryggvason, K., Sorokin, L., Fässler, R., Gu, G., Gerber, H.-P., Ferrara, N., Melton, D.A., Lammert, E.: The vascular basement membrane: A niche for insulin gene expression and beta cell proliferation. *Devel. Cell*, 10, 397-405, 2006.

Ordering information

PRODUCT	SUITABLE CELL TYPES	APPLICATIONS	QUALITY	PROD NO.	QUANTITY
Human Recombinant Laminin-511	Mouse and human ES iPS cells Other multipotent cells	Expansion of pluripotent ES, iPS cells and other multipotent cells	Non-GMP	50511 50512 50513	100 μ g 10 μ g 1000 μ g
Human Recombinant Laminin-411	Endothelial cells Insulin producing β -cells Neurons	Cardiovascular diseases Diabetes Alzheimer's Parkinson's	Non-GMP	50411 50412 50413	100 μ g 10 μ g 1000 μ g

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Application Note 004

Rapid single-cell suspension expansion of human ES and iPS cells without artificial inhibitors

Laminin-521

Culturing human embryonic stem (ES) cells in a controlled, biorelevant and repeatable manner is known to be difficult. Moreover, single-cell enzymatic passaging has been possible only by adding apoptosis inhibitors to the cell suspension. BioLamina now presents Laminin-521, a natural human ES cell niche protein that allows easy single-cell passaging and large-scale propagation of human ES and iPS cells.

Recombinant human Laminin-521 is a unique protein matrix that creates a biologically relevant environment to support pluripotent single-cell growth of human ES and iPS cells. In addition, handling human pluripotent stem cell cultures have never been easier and more standardized. Combined with an appropriate cell culture medium, this offers several key advantages:

1. Defined, xeno-free and clonal propagation of pluripotent human ES and iPS cells for months
2. Single-cell passaging for extremely easy and reliable passaging technique – concentrate on your experiments instead of cell culturing
3. No damaging rho kinase (ROCK) inhibitor needed for cell survival
4. Enzymatic single-cell passaging for automation and standardization
5. Biological relevance – Laminin-521 is the natural niche for human ES cells, and is expressed by pluripotent cells during early embryogenesis
6. Human recombinant Laminin-521 can be manufactured according to cGMP so you can take your innovation from bench to bedside

Defined clonal human ES and iPS cell expansion without artificial inhibitors

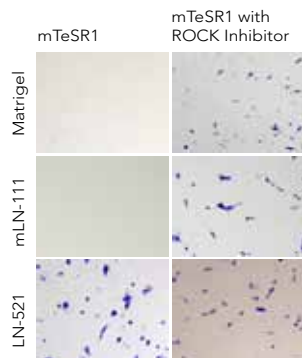


FIGURE 1: Survival of dissociated hES cells without ROCK inhibitor on human recombinant Laminin-521, mouse laminin-111 and Matrigel. Crystal Violet staining of hES cells 24 hours after single-cell suspension plating in mTeSR1 medium without and with Y-27632 treatment.

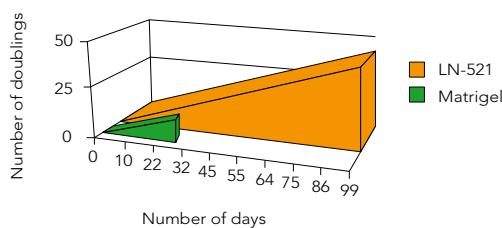


FIGURE 2: Human ES cell cultures expand rapidly and continuously for months when passaged as single-cells in the absence of ROCK inhibitor. Even better compared to cells grown on Matrigel passaged as clumps.

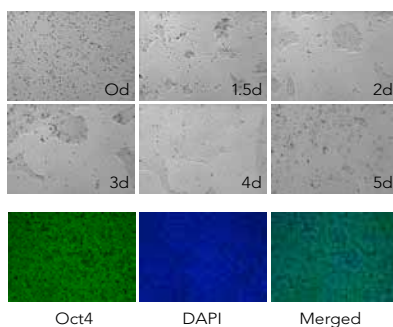


FIGURE 3: Pluripotent hES cells plated as single-cell suspension grow as a monolayer on top of Laminin-521. Laminin-521 mediates its effect via the $\alpha6\beta1$ integrin, signaling through the PI3K/Akt pathway and the $\alpha6\beta1$ integrin isoform is highly expressed in human ES cells. Laminin-521 has a very high affinity for $\alpha6\beta1$ and, thus, Laminin-521 can convey the highest dose of biorelevant signal, resulting in increased single-cell survival. The long-term expanded cells homogenously express Oct4 (green) and remain karyotypically normal.

REFERENCES

Rodin S et al. Poster #3540 (Abstract #1901) 9th ISSCR meeting June 15-19, 2011, Toronto, Canada.
 Rodin S, Niaudet C, Hansson EM, Domogatskaya A, Xiao Z, Nilsson AS, Holm F, Baker D, Inzunza J, Rozell B, Sun Y, Betsholtz C, Hovatta O, Tryggvason K.: LN-521 permits survival and expansion of dissociated human pluripotent cells through integrin $\alpha6\beta1$ -mediated migration and PI3K/Akt activation. Submitted. April 2011.

Ordering information

PRODUCT	SUITABLE CELL TYPES	APPLICATIONS	QUALITY	PROD NO.	QUANTITY
Human Recombinant Laminin-521	Mouse and human ES and iPS cells Other multipotent cells	Expansion of pluripotent ES, iPS cells and other multipotent cells	Non-GMP	50521 50522 50523	100 μ g 10 μ g 1000 μ g

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