APPLICATION NOTE 015

Human ES and iPS cell culture

On Biolaminin® 521 cell therapy grade

BioLamina

A cell culture substrate designed for clinical research

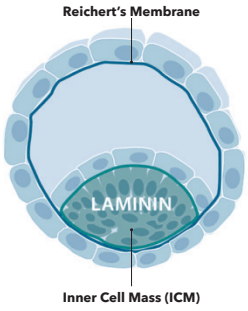
As a complement to our portfolio of defined and animal origin-free laminin stem cell substrates, we offer a cell therapy grade (CTG) Biolaminin 521 cell culture substrate for research-use or non-commercial manufacturing of cell, gene, or tissue-based products. Biolaminin 521 CTG (CT521) has been developed as material for use in the manufacturing of cells for clinical research. USP Chapter 1043: Ancillary materials for cell, gene, and tissue-engineered products has been considered in the design of the product. The product is animal origin-free to the secondary level in accordance to ISCT and has supporting documentation, such as Certificate of Analysis (CoA) and Animal Origin Free (AOF) Statement provided and is manufactured to allow customers to qualify the product.

Seamless transition from bench to clinic

CT521 is a full-length, human, recombinant laminin 521 substrate, the only one-of-a-kind on the market, providing an optimal environment for feeder-free culture of human pluripotent stem cells (hPSCs), mesenchymal stem cells (MSCs) and most anchorage-dependent progenitor cell types. With this cell therapy grade product, scientists are supported throughout their cell therapy development process – from concept to therapy.

Biologically relevant culture environment

Laminin 521 is a key basement membrane protein of the natural stem cell niche, expressed and secreted by hPSCs in the inner cell mass of the preimplanted embryo. Laminins bind to cell surface receptors activating cell signaling cascades, leading to more functional cells.



The CT521 substrate recreates a more authentic culture environment and supports reliable single-cell or colony expansion of hPSCs (both hESC and hiPSC).

Features and specifications:

- MX521 and CT521: cell therapy grade substrates
- For clinical research - designed considering USP Chapter 1043*
- Animal origin-free to the secondary level**
- Biologically relevant culture environment
- CT521: Includes extra quality documentation

- Manufacturing control and traceability
- Consistent and reliable cell performance
- Easy and flexible culture system
- Homogenous and genetically stable hPSC cultures

Direct link to Biolaminin 521 CTG information online

*U. S. Pharmacopeia. Chapter 1043: Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
** ISCT guidance document "ISCT Animal-Free Origin Survey Results-Summary"

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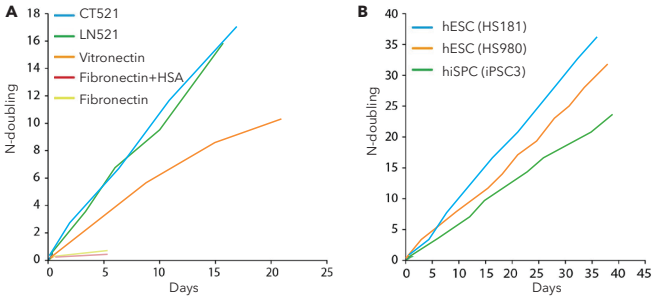
FIGURE 1

Rapid and stable hPSC expansion on CT521

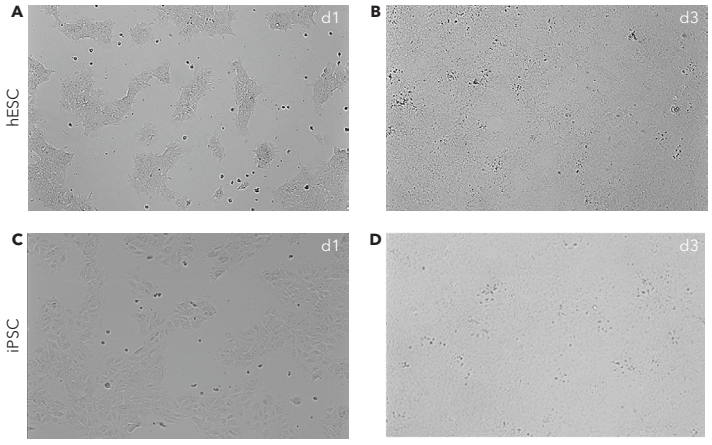


A) hPS cells passaged as single cells (w/o ROCKi) on CT521 and LN521 in iPS-Brew medium have similar proliferation rate and accumulate significantly faster compared to cells cultured on Vitronectin. Fibronectin substrates could not support cell growth.

B) Population doubling (10 passages) for hES cell lines HS181 and H980 and hiPS cell line iPSC3.

FIGURE 2

Homogenous hPSC monolayer on CT521

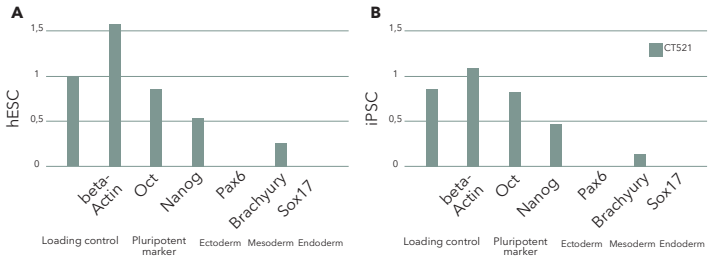


- Single-cell seeding
- Derivation & gene editing
- No ROCKi needed
- Monolayer growth
- No spontaneous differentiation
- Compliant with any medium

A, B) hES (HS181) and C, D) hiPS (iPSC3) cells exhibit a normal morphology at day 1 (d1; A, C) and after growing to near confluency at day 3 (d3; B, D) on CT521.

FIGURE 3

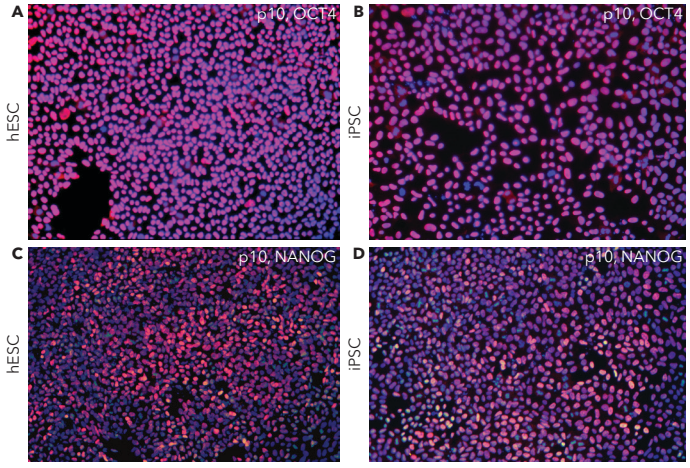
Pluripotency and differentiation marker expression in hESC and hiPSC



A) hES (HS181) and B) hiPS (iPSC3) cells express the pluripotent markers Nanog and Oct4. Negative ectoderm (Pax6) and endoderm (Sox17) marker expression, and only slight mesoderm expression (Brachyury).

FIGURE 4

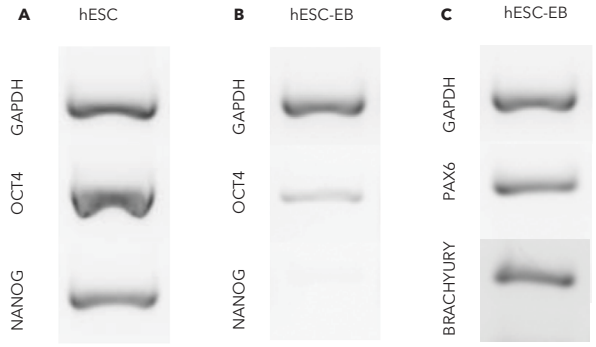
Pluripotent protein expression of hESC and hiPSC on CT521



A,C) hES (HS181) and B, D) hiPS (iPSC3) cells cultured on CT521 as single-cells in iPSC-Brew medium for 10 passages remain pluripotent, confirmed by Immunocytochemistry for OCT4 (A-B; pink), NANOG (C-D; pink) and DAPI (A-D; blue).

FIGURE 5

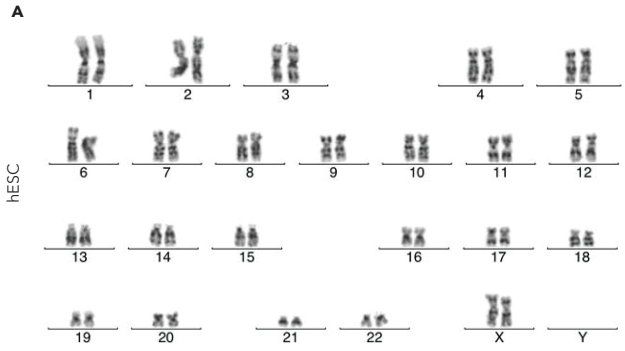
hPSC show multipotency with embryoid body (EB) formation after long-term culture on CT521



A) hES (HS181) cells cultured on CT521, maintain the protein pluripotent markers NANOG and OCT4 when cultured as single cells for 10 passages. B, C) Upon EB formation, the pluripotent marker (B, NANOG and OCT4) protein expression decreases, and ectoderm (C, PAX6) and mesoderm marker expression (C, BRACHYURY) is upregulated.

FIGURE 6

Normal karyotype maintenance of hPSC



A) hES cells (HS181) present a normal karyotype after 13 passages on CT521.

REFERENCES

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