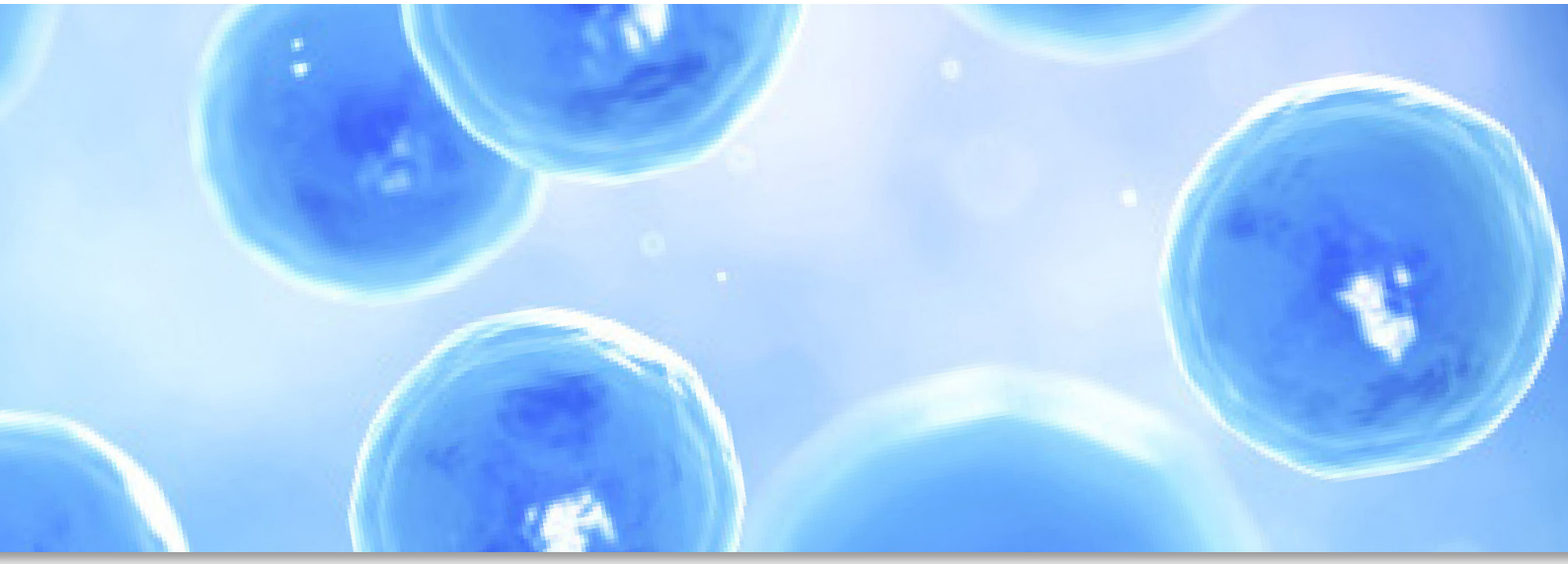
BioLamina
REVOLUTIONIZING CELL CULTURE

APPLICATION NOTE 001

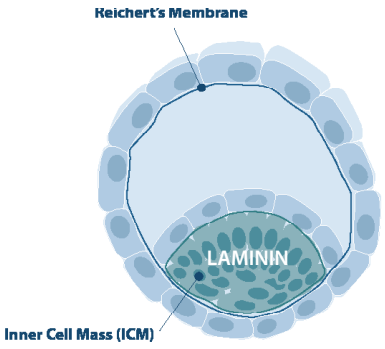
WEEKEND-FREE FEEDING PROTOCOL

CULTURE OF HUMAN PLURIPOTENT STEM CELLS ON LN521



WITH THE BIORELEVANT SUPPORT FROM THE LN521 CELL CULTURE MATRICE, HUMAN ES AND iPS CELLS CAN BE CULTURED WITHOUT WEEKEND FEEDING

Laminin 521 is a key cell adhesion protein of the natural stem cell niche. It is expressed in the basement membranes of the inner cell mass in the developing embryo and secreted by human pluripotent stem cells (hPSCs) in culture. The defined and animal origin-free Biolaminin 521 LN (LN521) cell culture matrix creates a biologically relevant culture environment that makes handling of human ES and iPS cells easy, reliable and standardized. The cells grow in a homogenous monolayer without any need for manual removal of differentiated cell areas. Easy and controlled single-cell passaging can be performed both with and without enzyme and is totally independent of apoptosis inhibitors, such as ROCKi.



Most established hPSC culture protocols require daily medium replacement, demanding researchers to routinely work weekends. Based on the biorelevant support from LN521, we developed a protocol that eliminates feeding on Saturdays and Sundays. Your hPSC cultures will maintain characteristic cell morphology, high pluripotent cell marker expression and high expansion rates. The protocol is flexible - the only important guideline is that the cells must be passaged at the end of the week (Thursday or Friday) to ensure low cell density during the weekend.

FEATURES AND SPECIFICATIONS:

- **Defined and animal origin-free (primary level) substrate**
- **Biologically relevant hPSC culture environment**
- **No feeding required Saturdays or Sundays – Work-free weekends!**
- **The cells maintain high pluripotency expression and proliferation rate**
- **Easy, efficient and reliable single-cell passaging enables standardized experiments**
- **The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.**
- **Protocol validated with low bFGF concentration medium**
- **For research use only**

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Direct link to Biolaminin 521 LN information online

LN521 FACILITATES RELIABLE LONG-TERM SELF-RENEWAL OF HUMAN PLURIPOTENT STEM CELLS WITHOUT WEEKEND FEEDING

We validated the weekend-free feeding protocol (feeding only weekdays) for support of long-term maintenance of human ES and iPS cells by comparing it to a standard, everyday feeding protocol (feeding 7 days/week). In both cases, cells were routinely passaged as single cells twice/week but always with a passage at the end of the week (Thursday or Friday). For more information about the LN521 single-cell passage protocol, see **INSTRUCTIONS FOR USE 003**. Experiments were performed using two hES cell lines (HS181 and HS980) and one iPS cell line (C3), maintained in Nutristem XF medium on LN521 stem cell matrix for 6 passages. Both culturing protocols were performed in parallel with 4 independent wells (n=4) for each cell line and protocol.

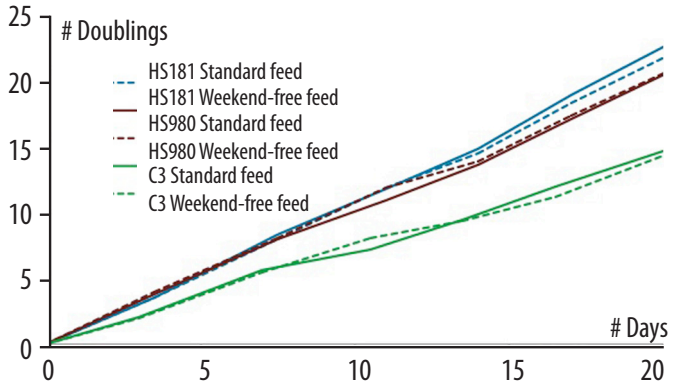

Figure 1. Cell expansion rates are just as high under weekend-free feeding condition
Human ES cells (HS181 and HS980) or iPS cells (C3) were maintained on LN521 in Nutristem under the standard, every day-feeding (filled lines) or weekend-free feeding conditions (dashed lines) for 6 passages. Fold expansion at each passage was determined by comparing the number of cells generated to the amount seeded.

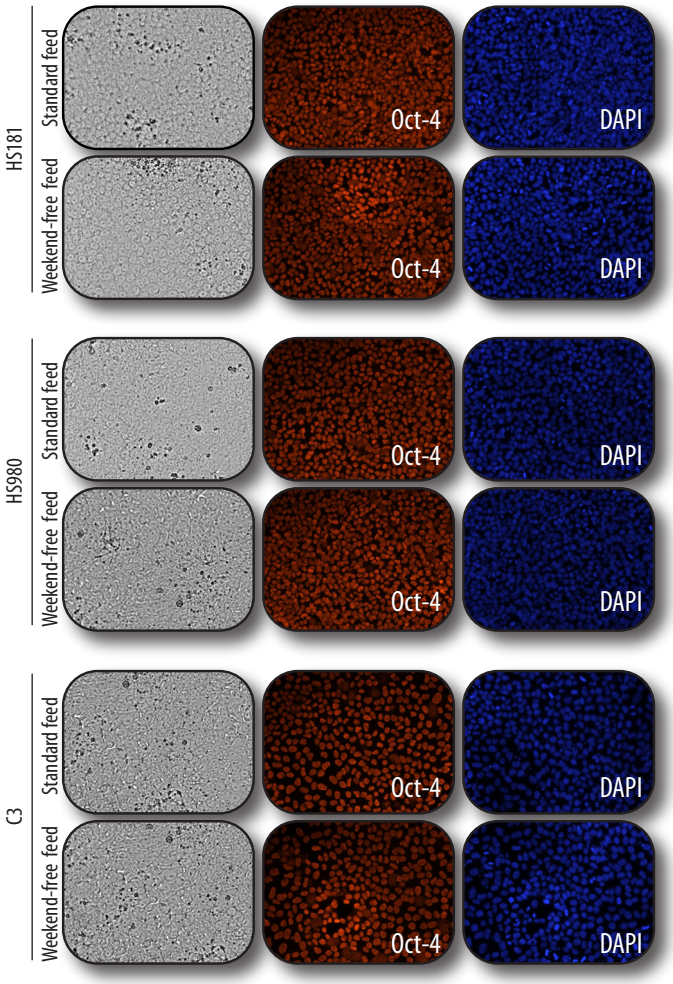

Figure 2. Weekend-free cultured cells maintain characteristic, pluripotent cell morphology and protein expression.
Expression of pluripotent stem cell marker OCT-4 (red staining) was high after 6 passages for both the human ES cells (HS181 and HS980) and iPS cells (C3) maintained on LN521 in Nutristem using standard or weekend-free feeding protocols. DAPI staining (blue) was used as control. The cells also consistently maintained undifferentiated cell morphology (bright-field pictures).

Compared to the standard protocol, all three cell lines cultured with the weekend-free culture protocol maintained the same high, long-term expansion rate for 6 passages (Figure 1). Independent of culture protocol, all three cell lines grew as a homogenous monolayer with characteristic hES and iPS cell morphology with prominent nucleoli and a high nuclear to cytoplasmic ratio (Figure 2, bright-field pictures). The cells also maintained the features of undifferentiated human ES and iPS cells as illustrated by high expression of the pluripotent stem cell marker OCT-4 (Figure 2, red staining).

REFERENCES

- Higher-Density Culture in Human Embryonic Stem Cells Results in DNA Damage and Genome Instability. Jacobs et al. Stem Cell Reports, 2016
- Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Rodin et al. Nat Commun. 2014

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