

## WITH AN EASY, EFFICIENT AND RELIABLE SINGLE CELL PASSAGE ON LN-521™, IT IS POSSIBLE TO MAINTAIN HUMAN ES OR IPS CELL CULTURES WITHOUT WEEKEND FEEDING

Most established human pluripotent stem cells (hPSC) culture protocols require daily medium replacement, demanding researchers to routinely work weekends. Based on the biorelevant support from laminin-521 (LN-521), we developed a protocol that eliminates feeding on Saturdays and Sundays. The protocol is flexible - just feed your cells daily during weekdays and perform a simple LN-521 single cell passage on Thursday or Friday. Splitting frequency will be cell line dependent, therefore simply adjust the seeding density, optimal for your cell line and work schedule. The only important guideline is that the cells must be passaged at the end of the week (e.g. Thursday or Friday). Your pluripotent stem cell (PSC) cultures will maintain characteristic cell morphology, high pluripotent cell marker expression and high expansion rates.

## LN-521 STEM CELL MATRIX - A BIORELEVANT, CHEMICALLY DEFINED AND XENO-FREE, PLURIPOTENT CELL CULTURE SYSTEM

Laminins are the most abundant component of the basement membranes, which all cells interact with. There are 16 distinct laminin isoforms identified, with a tissue-specific distribution. Laminin-521 is the first extracellular protein expressed during development and is secreted by the pluripotent stem cells in the blastocyst inner cell mass. By coating your cultureware with LN-521 stem cell matrix, you successfully recreate the natural stem cell niche.

The biorelevance of LN-521 makes handling of hPSC easy, reliable and standardized. The cells grow in a homogeneous monolayer without any need for manual removal of differentiated cell areas. The robust support of LN-521 makes long-term self-renewal of hPSC without artificial apoptosis inhibitors possible, and enables a totally defined and xeno-free cell culture system. We have successfully and efficiently grown hESC and iPSC for more than 100 passages with maintained pluripotency and genetic integrity.



### KEY ADVANTAGES

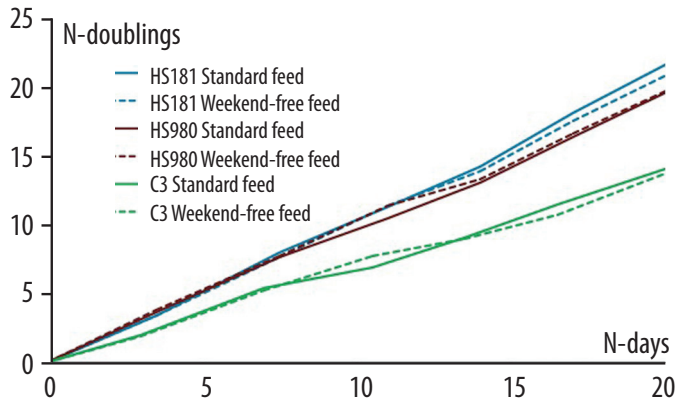
- **No feeding required Saturdays or Sundays – Work-free weekends!**
- **No strict passaging schedule**
- **The cells maintain high pluripotency expression and proliferation rate**
- **Easy, efficient and reliable single cell passaging enables standardized experiments**
- **Defined and xeno-free long-term propagation of hPSC**
- **Protocol validated with a low bFGF concentration medium**



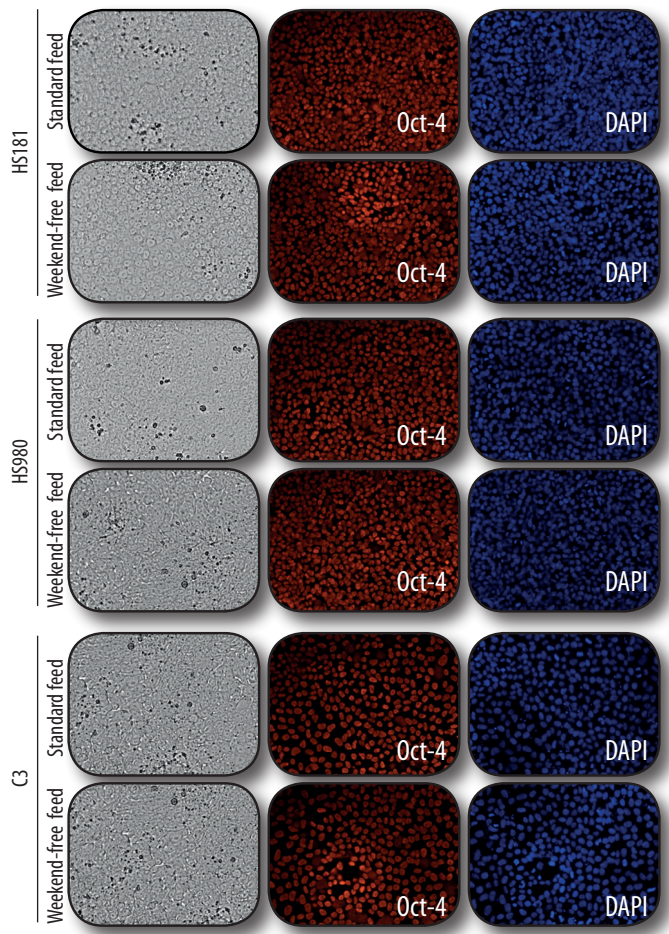
Direct link to LN-521™  
information online

# LN-521 FACILITATES LONG-TERM SELF-RENEWAL OF HUMAN PLURIPOTENT STEM CELLS WITHOUT WEEKEND FEEDING

We validated the weekend-free feeding protocol (feeding only week-days) for support of long-term maintenance of human ES and iPS cells by comparing it to standard, every day 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 easy and reliable LN-521 single-cell passage protocol, see **INSTRUCTIONS FOR USE BLO03**. Experiments were performed using two hES cell lines (HS181 and HS980) and one iPS cell line (C3), maintained in Nutristem XF medium on LN-521 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 in Nutristem under 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 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).

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