Single cell passaging with NutriStem® hPSC XF on laminin-521 without the need for ROCK inhibitor

Introduction

Human Pluripotent Stem cells (hPSC) are of great potential for cell therapy and regenerative medicine in many severe diseases. Removing xeno components and using feeder-free cultures is necessary to ensure robust and reproducible colonies during culture. Passaging as small and large aggregates using reagents such as collagenase or EDTA meet these xeno-free conditions, however they are not always compatible with desired downstream applications such as gene editing or CRISPR/Cas9 applications. As a result, single cell passaging is becoming a standard method in today's cell culture practices.

Utilizing a completely dissociated single cell suspension for passaging generates a monolayer culture that has advantages of higher culture scalability, rapid expansion, and high efficiency. In addition, because of its quantitative nature, single cell suspension makes expansion of hPSC reliable and standardized. However, single cell passaging places the hPSC in stressful conditions, resulting in increased cell death. Usually, this requires the addition of a small anti-apoptotic molecule Y-27632, a selective inhibitor of Rho-associated coiled-coil kinase (ROCK), to avoid apoptosis and to increase hES cloning efficiency from ~1% to ~27% [1].

The combination of xeno-free media (i.e. NutriStem® hPSC XF, cat.# 05-100-1), recombinant protein matrices (i.e. LaminStem™ 521, #cat 05-753-1) and recombinant trypsin solutions (i.e. Recombinant Trypsin EDTA Solution, cat.# 03-079-1) enables single cell passaging without the addition of ROCK inhibitor, while maintaining cell integrity and characteristics. With the support from the laminin-521 matrix, cells can be passaged in very low cell densities and can be cultured to high confluence without phenotypic alterations[2].

When used with NutriStem® hPSC XF medium, LaminStem™ 521 promotes cellular survival and expansion of hPSC after plating from single-cell suspension. When cultured with LaminStem™ 521, the hPSC grow as a monolayer and remain pluripotent with minimal spontaneous differentiation.

In the following section we will highlight a user-friendly protocol for single-cell passage of hPSC grown in xeno-free conditions with NutriStem® hPSC XF and Laminin 521.

Protocol

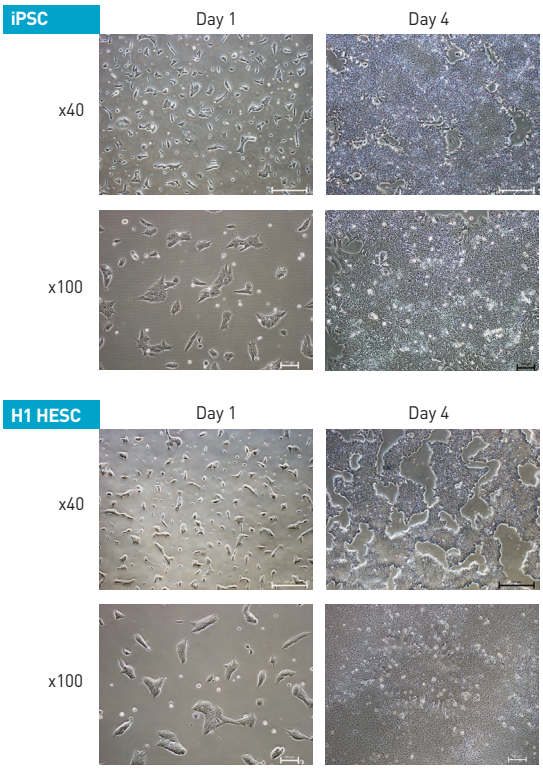
This procedure describes the passage of hPSC from 1 well of 6-well plate.

1. Wash cells twice with 2ml/well DPBS (BI, cat.# 02-023-1) without calcium and magnesium
2. Add 1ml of Recombinant Trypsin EDTA Solution (BI, cat# 03-079-1)
3. Incubate at 37°C for 2-4 minutes. Exposure time may vary and should be adjusted. Note: Do not over-expose the cells to the Recombinant Trypsin-EDTA Solution
4. Detach and break up colonies by pipetting 5-6 times up and down with a 1ml pipette. Make sure the pipetting washes the entire well
5. Add 4ml 1xSBTI (Soybean Trypsin Inhibitor (50X) 5mg/ml, BI, cat# 03-048-1) to the Recombinant Trypsin-EDTA Solution
6. Collect cells into a new sterile centrifuge tube
7. Centrifuge at 200g for 5 minutes
8. Suspend the pellet with 1 ml of NutriStem® hPSC XF medium
9. Count cells
10. Plate cells at the desired density in Laminin 521 pre-coated wells with 3-4ml pre-equilibrated medium. Usually, 10-20,000 cells/cm^2 for splitting every 4-5 days
11. Place the plate in a 37°C 5% CO_2 incubator. Move the plate several times back and forth and side to side to evenly distribute the cells in the well
12. Do not move the plate during the first 48 hours post-split. Perform daily medium change after 48 hours with 2.5-3.0ml/well NutriStem® hPSC XF until the culture is nearly confluent (60-70%) and ready for passage

Notes:

1. It is critical not to move the seeded plates during the first 48 hours after splitting as this may increase differentiation of hPSC.
2. Cells are ready to be passaged when culture is ≥60% confluent. Optimal seeding density will vary from one cell line to another and must be determined for your system. With optimal culture conditions and seeding density, most cell lines will reach confluency within 4-5 days and expand 10-25-fold.
3. It is possible to perform higher volume feed (4-5ml) for the weekend and skip 2 days without changing the medium. In this case, the cells must be passaged at the end of the week.
4. Optimal Laminin 521 coating concentration is cell-dependent and should be calibrated. 0.5-1 µg/cm^2 should work well for most hPSC.
5. Use higher cell density (40-80,000/cm^2) for the first few passages, until the cells are fully adapted to the Laminin 521 coating and the single cell dissociation.

Single cell passage using Recombinant Trypsin EDTA Solution and NutriStem® hPSC XF



Typical recovery of ATCC-DYP0530 IPSC (P26) and H1 (61) hESC from single-cell passage using recombinant Trypsin EDTA solution and NutriStem® hPSC XF medium on 0.5µg/cm² Laminin 521. Representative images for colony morphology one day and 4 days post-seeding.

Summary

Whether to promote cell viability and get more cells (per cm²) than usual or to perform gene editing on your hPSC, a method for growing cells from single-cell cultures is needed. Now, with the combination of NutriStem® hPSC XF and Laminin521, single cell passaging is possible without the addition of ROCK inhibitor but with the same added value to the cells' survival and viability. The hPSC will grow as a monolayer and remain pluripotent with minimal spontaneous differentiation.

Products

Cat. #	Product	Qty
05-100-1A	NutriStem® hPSC XF Medium	500 ml
05-753-1F	LaminStem™	1 ml
03-079-1	Recombinant Trypsin EDTA Solution	100 ml
03-048-1C	Soybean Trypsin Inhibitor (50X)	5mg/ml 20 ml

Further reading

- Maroof M Adil, David V Schaffer. *Expansion of human pluripotent stem cells.* Current Opinion in Chemical Engineering 2017, 15:24–35
- R. Bergström, Xeno-free culture of human pluripotent stem cells, Methods Mol Biol. 2011;767:125-36
- K. Jacobs et al. *Higher-Density Culture in Human Embryonic Stem Cells Results in DNA Damage and Genome Instability.* Stem Cell Reports: 6(3), pp 330–341, 2016
- Y Qin, et al. *Laminins and cancer stem cells: partners in crime?* Seminars in Cancer Biology, 2016
- O. Simonson. Use of Genes and Cells in Regenerative Medicine. Karolinska Institutet, 2015
- S. Rodin et al., *Monolayer culturing and cloning of human pluripotent stem cells on laminin-521–based matrices under xeno-free and chemically defined conditions.* Nature Protocols 9, 2354–2368 (2014) doi:10.1038/ nprot.2014.15
- Rodin S, et al. *Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment.* Nat Commun. 5:3195. doi: 10.1038/ ncomms4195, 2014

Clinical Applications

- Hovatta, Outi. *Infectious problems associated with transplantation of cells differentiated from pluripotent stem cells.* Seminars in Immunopathology: Volume 33, Issue 6, pp 627-30, April 2011

References

1. Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, et al. (2007) A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol 25: 681–686. Rodin, Sergey, Anna Domogatskaya, Susanne Ström, Emil M. Hansson, Kenneth
2. R. Chien, José Inzunza, Outi Hovatta, and Karl Tryggvason. *2010. "Long-Term Self-Renewal of Human Pluripotent Stem Cells on Human Recombinant Laminin-511."* Nature Biotechnology 28 (6): 611–15.

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