Novel precoating-free protocol for culturing hPSC using vitronectin

Introduction

Human Embryonic Stem Cells (hESCs) and human induced pluripotent stem cells (iPSCs) can proliferate long-term without losing the ability to maintain pluripotency. Thus expanding in a large number of stem cells, where each has the capability to differentiate into any cell or tissue in the body. These characteristics make hESCs and iPSCs an exceptional tool for disease modelling and cell-based therapies.

The culturing of hESCs and iPSCs and maintaining their undifferentiated state initially depended mainly on the support of feeder cells such as mouse embryonic fibroblasts (MEFs), or extracellular matrices such as Matrigel™. However, the xenogeneic components of these undefined systems increases inconsistency, variability and the risk of contamination with adventitious agents.

The use of feeder-free systems makes it possible to culture hESCs and iPSCs cells in a xeno-free and chemically defined environment. This has many advantages, such as consistency in the formulations that prevent lot-to-lot variability, thus ensuring a process that is reliable, reproducible, robust and safe.

The development of a clinically compliant hESCs/iPSCs culture model requires a complete defined, xeno-free culture system, in which the culture medium as well as the cell culture matrix are defined. A purified attachment protein like recombinant vitronectin has appeared as a suitable matrix/substrate for long-term culture of hESCs/iPSCs in defined (or serum-free or xeno-free) conditions[1].

Biological Industries has developed NutriStem® V9 XF (composed of basal medium cat.# 05-105-1A and supplement cat.# 05-106-1F), a defined, xeno-free, serum-free medium specially formulated to support the growth and expansion of hPSC using vitronectin and enzyme-free passage with EDTA. NutriStem® V9 XFmedium allows a high proliferation rate and long-term culture, while maintaining stable karyotype, high pluripotency marker expression, and tri-lineage differentiation potential of hESCs/iPSCs.

In the following section we will discuss a user-friendly, time-saving protocol that has been developed to eliminate the traditional coating procedure. While seeding, Vitronectin ACF (BI, cat. # 05-754-0002) is added directly into NutriStem® V9 XF medium, making precoating unnecessary.

Novel Precoating-free protocol

In this friendly-to-use, time-saving procedure Vitronectin ACF (BI, cat. # 05-754-0002) is added directly into pre-equilibrated NutriStem® V9 XF medium prior to hPSC seeding. The novel precoating-free procedure will require only 0.25-0.375µg/cm² of Vitronectin ACF. Optimal concentration is cell dependent and should be calibrated; it is recommended to test 25-50% less Vitronectin ACF than the concentration used in the standard pre-coating procedure.

Novel precoating-free procedure	Vitronectin ACF coating concentration	0.25-0.375µg/cm²	→ **~30** minutes
	Vitronectin ACF volume required*	5-7.5µl	
Traditional coating procedure	Vitronectin ACF coating concentration	0.5µg/cm²	→ **90** minutes minimum
	Vitronectin ACF volume required*	10µl	

* From 0.5mg/ml solution

Precoating-free protocol

(1 well in a 6-well tissue culture plate)

1. Thaw Vitronectin ACF on ice
2. Add 3ml/well of complete **NutriStem® V9 XF** medium
3. Equilibrate for at least 30 minutes in a 37°C CO_2 incubator
4. Harvest cells according to the procedure described below
5. Add 5-7.5µl of Vitronectin ACF to the 3ml pre-equilibrated NutriStem® V9 XF medium and swirl
6. Perform enzyme-free cell passage according to the procedure described below
7. Plate the cell aggregates at the desired density in the 3ml complete **NutriStem® V9 XF** medium containing the Vitronectin ACF. Usually a splitting ratio of 1:8-1:20 every 4 days is required
8. Place the plate in a 37°C CO_2 incubator. Move the plate several times back and forth, and side to side to distribute the aggregates evenly in the well
9. After 48 hours, change the medium daily with 3ml/well complete **NutriStem® V9 XF** medium until the colonies are large enough to passage

Note: Vitronectin ACF may also be added before NutriStem® V9 XF pre-equilibration.

Results

Morphology

Typical undifferentiated hPSC colony morphology is maintained during long-term expansion (P11 and P25) in NutriStem® V9 XF using both traditional coating and the novel precoating-free culture procedure (figure 1).

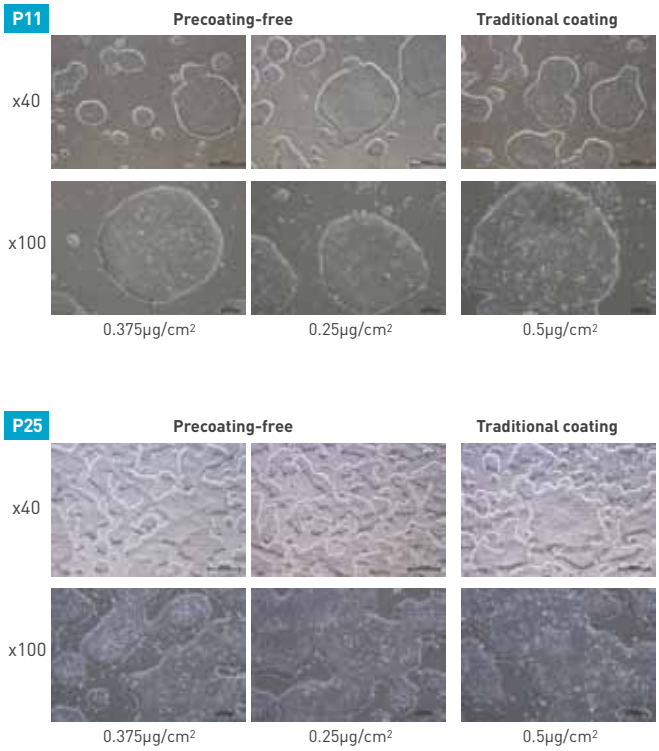

Figure 1: Colony morphology of H1 hESC cultured in NutriStem® V9 XF for P11 and P25 sequential passages using traditional coating and novel precoating-free procedure. When working according to the precoating-free protocol Vitronectin ACF is added directly to NutriStem® V9 XF culture medium before cells seeding.

Proliferation

NutriStem® V9 XF supports high proliferation rate during long-term culture of hPSC using both traditional coating and novel precoating-free culture procedure (figure 2).

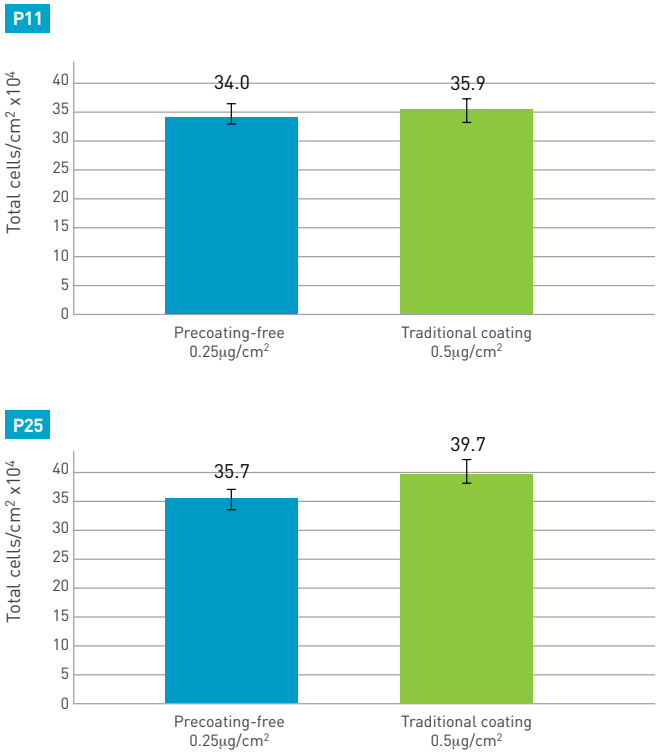

Figure 2: Nucleocounts of H1 hESC expanded for P11 and P25 sequential passages in NutriStem® V9 XF on Vitronectin ACF matrix using both novel precoating-free and traditional coating procedure. Every 3-5 days cells were passaged as small aggregates using 0.5mM EDTA solution. Results presented as fold of expansion (total cells/initial seeded).

Immunophenotyping

NutriStem® V9 XF supports high expression of pluripotent stem cell markers in hPSC expanded using precoating-free procedure (figure 3).

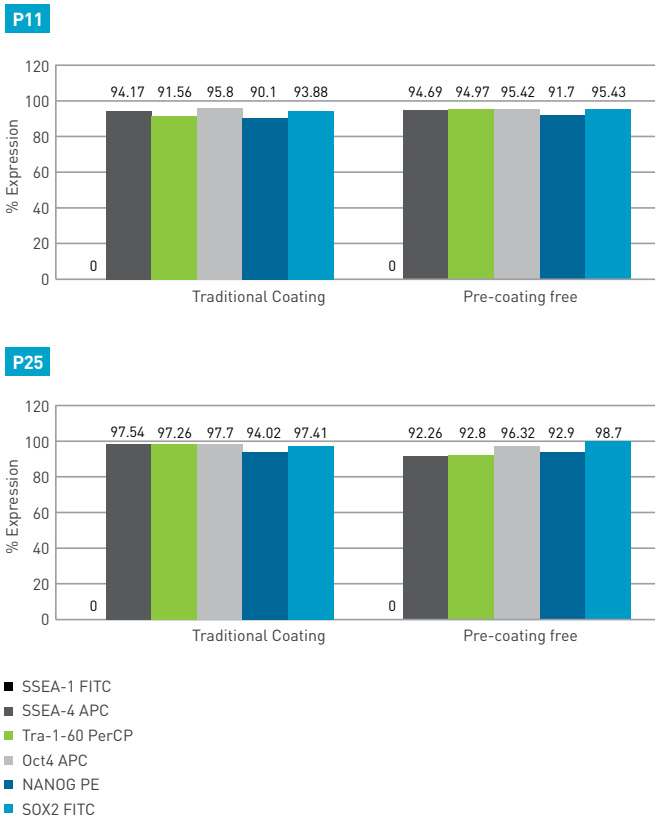

Figure 3: Flow cytometry analysis of H1 hESC culture expanded in NutriStem® V9 XF for P11 and P25 sequential passages using traditional coating and novel precoating-free procedure. At the indicated passage cells were analyzed for pluripotent marker expression by flow cytometry. Data presented as % expression from gated viable cells.

Immunofluorescence staining

High levels of pluripotent markers maintained in hPSC expanded in NutriStem® V9 XF using the novel precoating-free procedure.

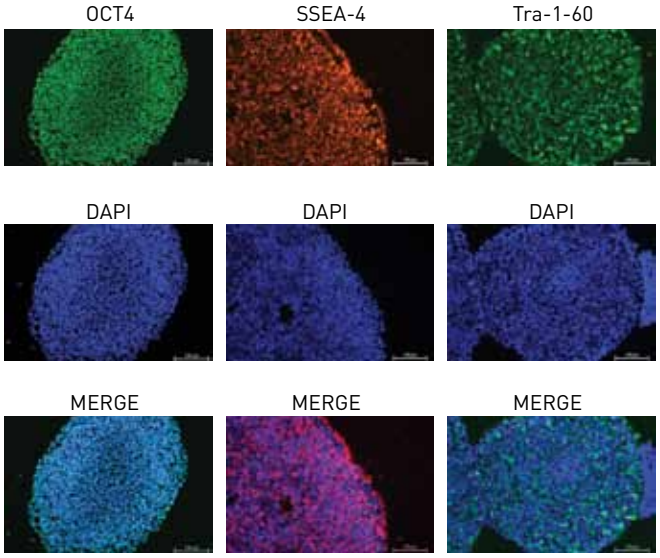

Figure 4: Immunofluorescence analysis of human pluripotent markers of H1 hPSC expanded in NutriStem® V9 XF medium using the precoating-free for 8 passages. Cells from P8 were fixed and stained for the classical pluripotent surface markers: SSEA-4 (RRX) (red), TRA-1-60 (Alexa fluor) (green) and nuclear conjugated markers: OCT-4-Alexa fluor, counterstained with DAPI (blue). Scale bar 200 µm.

Conclusion

In a world of "time is money", any innovation that saves time and is user-friendly is appreciated. The option of removing the pre-coating requirement is a necessary advancement in the scientific world, where results are expected to come sooner rather than later. The precoating-free option enabled by the combination of NutriStem® V9 XF and vitronectin ACF, and the supporting evidence that the culture is equivalent to precoated cultures, is a definite advantage to labs and companies seeking to streamline their research and/or production.

Products

Product Name	Cat. No.	Size
NutriStem® V9 XF Medium Basal Medium	05-105-1A	500ml
NutriStem® V9 XF Supplement Mix	05-106-1F	1ml
Vitronectin ACF	05-754-0002	200 µg
0.5M EDTA solution	01-862-1B	100mL

References

1. Desai, Nina, Pooja Rambhia, and Arsela Gishto. "Human embryonic stem cell cultivation: historical perspective and evolution of xeno-free culture systems." *Reproductive Biology and Endocrinology* 13.1 (2015): 9.

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