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PROTOCOL

Efficient Transition of Human Pluripotent Stem Cell Cultures from Essential 8™ Medium into NutriStem® hPSC XF Medium on Vitronectin

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Introduction

Establishing ideal culture methods has been a known challenge in human pluripotent stem cell (hPSC) research since the creation of the first embryonic stem cell line in the late 90s and the discovery of induced pluripotent stem cells in 2006. Traditional protocols often call for highly variable components such as cell feeder layers, due to their ability to aid cell maintenance through secretion of essential growth factors, ECM components, and cytokines including bFGF, TGF-β, laminin, and others.[1] However, as the need for more defined cultures becomes increasingly prevalent, carefully formulated microenvironments have since evolved for more efficient maintenance of cellular pluripotency and purity, in turn reducing the use of feeder layers. Ultimately, as standards for clinical-quality cell use in therapeutic applications are determined, media formulations and culture conditions must be refined and optimized while fully supporting proper phenotype and genetic stability.

Today, for clinical translation of hPSCs into cellular therapy, the recommendation is to culture and expand these cells in xeno-free environments that oftentimes utilize recombinant human cell culture substrates like vitronectin. One option for xeno-free culture when using vitronectin is Essential 8™ Medium (E8), but when compared to Biological Industries' NutriStem® hPSC Medium, E8 contains extensive amounts of growth factors with the intention of maintaining pluripotency and preventing differentiation.[2] However, it has been shown that high amounts of growth factors also play a critical, and sometimes detrimental, role in regulating the downstream differentiation of hPSCs into mature cell types. For example, researchers have shown that high concentrations of bFGF can inhibit early neural differentiation of hPSCs into PAX6+ neuroectodermal cells yet are beneficial in promoting more mature neuronal differentiation of lineage committed early neural precursors.[3]

Considering both the positive and negative downstream effects related to daily exposure of hPSCs to high concentrations of growth factors, an ideal cell culture medium should contain low amounts of growth factors that can be controlled and supplemented to higher levels as needed, rather than subjecting cells to high amounts of growth factors that cannot be reduced.

NutriStem hPSC Medium has been optimized with minimal amounts of bFGF while still maintaining pluripotency, allowing hPSCs to expand efficiently on a variety of substrates (Matrigel®, vitronectin, and laminin) with minimal differentiation. However, when transitioning from a high bFGF-containing medium, such as E8 (100 ng/mL), to a low bFGF medium like NutriStem hPSC Medium (<10 ng/mL), users should be aware that they may notice initial differences in hPSC colony appearance and morphology (such as less compact colonies with less defined borders).

This protocol is the suggested method for adapting hPSCs originally cultured in E8 on vitronectin to NutriStem hPSC Medium on vitronectin. By directly adapting and supplementing NutriStem hPSC Medium with an additional 100 ng/mL of bFGF, similar E8 morphological features can be retained, while supporting higher levels of pluripotency. Critically, this protocol facilitates the transition to a completely xeno-free culture system (NutriStem hPSC Medium + vitronectin) that results in more stable (minimal spontaneous differentiation) and pluripotent long-term hPSC cultures.

Adaptation Protocol

Generally, there are two options for transitioning cells from one medium to another: direct or gradual adaptation. With direct adaptation active cultures are switched from the first medium directly into the next all at once, in a single medium exchange. Alternatively, with gradual adaptation, active cultures are switched from the first medium into the next in several steps. Gradual adaptation tends to be gentler on cells than direct adaptation, but its necessity should be determined on a case-by-case basis.

To minimize the morphological differences that occur when transitioning cells from E8 to NutriStem hPSC Medium, cultures may benefit from:

1. Supplementing NutriStem hPSC Medium with an additional 100 ng/mL of bFGF
2. Performing a direct adaptation into NutriStem hPSC Medium

Protocol

Transition (7 Days)	**DAY 0**	Begin with active hPSC cultures
	DAYS 1-4	100% E8
	DAY 5	100% NutriStem hPSC Medium (+100 ng/mL bFGF) (completely replace E8)
	DAY 6	100% NutriStem hPSC Medium (+100 ng/mL bFGF) (change media)
	DAY 7	Passage hPSC cultures onto fresh vitronectin in 100% NutriStem hPSC Medium (+100 ng/mL bFGF)
Stabilized (15-20 Days)	**DAYS 7-28**	Passage hPSC cultures every 4-5 days during the stablization process and feed daily with 100% NutriStem hPSC Medium (+100 ng/mL bFGF)
	DAY 28+	Downstream experiments (scale-up, gene editing, differentiation)

Note: To appropriately supplement NutriStem hPSC Medium with added bFGF, reconstitute the bFGF as per the manufacturer's instructions, add required volume of bFGF to the medium, and gently mix before use.

Morphology Comparison

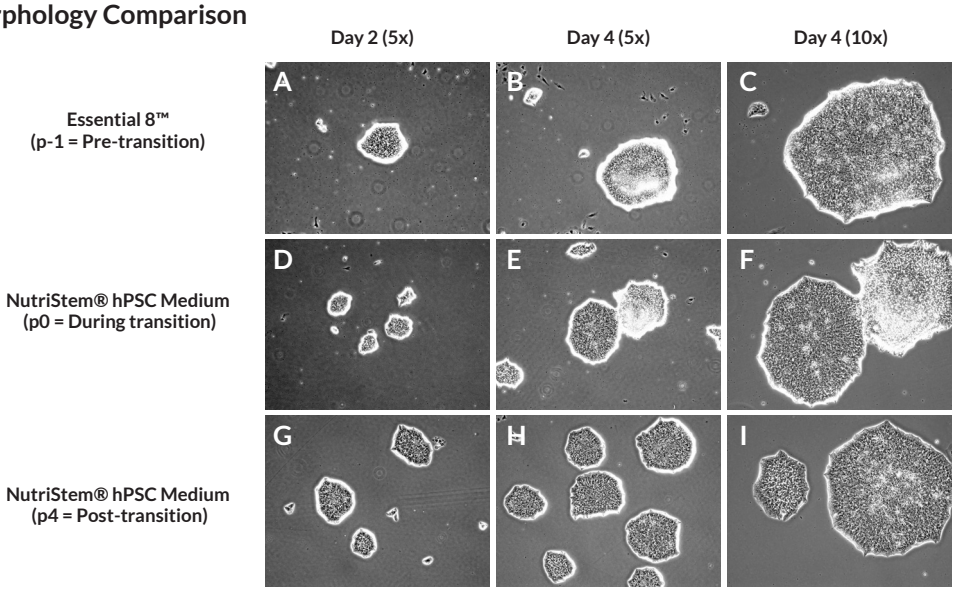

Figure 1. hPSC cultures on vitronectin transitioned from E8 to NutriStem hPSC Medium exhibit similar morphologies. A morphological comparison of hPSC cultures before, during, and after transition from E8 to NutriStem hPSC Medium demonstrates small colonies with moderate levels of spontaneous differentiation in E8 (Fig. 1, A-C), similarly small colonies with less differentiation and a more dome-like appearance that flatten out over time upon initial transition to NutriStem hPSC Medium (Fig. 1, D-F), and faster expanding, more compact colonies with tight colony borders once stabilized in NutriStem hPSC on vitronectin for 4 passages (Fig. 1, G-I). Overall, the level of spontaneous differentiation in NutriStem hPSC Medium was less than cultures maintained in E8 (data not shown). All cultures in NutriStem hPSC Medium were supplemented with an additional 100 ng/mL of bFGF and directly transitioned (as outlined in the Protocol).

Pluripotency Comparison

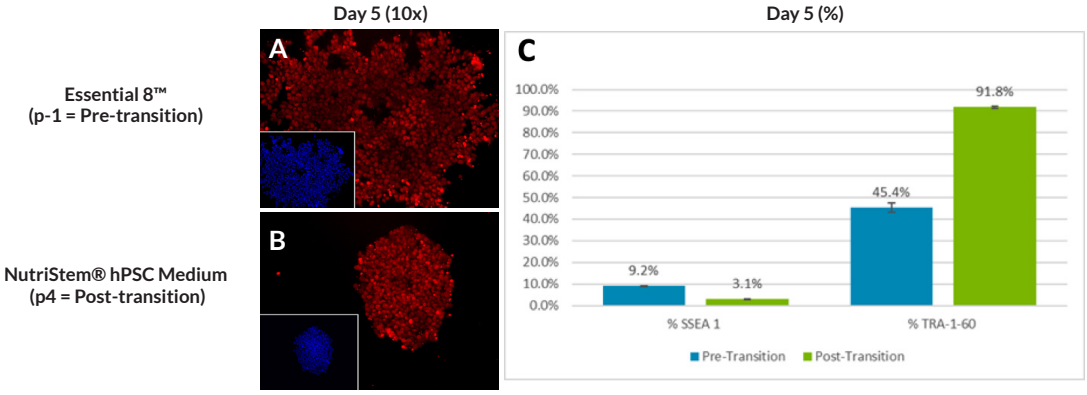

Figure 2. hPSC cultures on vitronectin transitioned from E8 to NutriStem hPSC Medium exhibit higher levels of pluripotency marker expression. Pluripotency was measured both pre- and post-transition from E8 to NutriStem hPSC Medium (with additional 100 ng/mL bFGF added). Prior to the transition, active hPSC cultures in E8 were assessed via immunocytochemistry for the nuclear pluripotency marker Nanog (Fig. 2, A) as well as by flow cytometry for SSEA1 and TRA-1-60 (Fig. 2, C). Following transition of hPSCs to NutriStem hPSC Medium, cultures were also assessed via immunocytochemistry for the nuclear pluripotency marker Nanog (Fig. 2, B) as well as by flow cytometry for SSEA1 and TRA-1-60 (Fig. 2, C) demonstrating that hPSC cultures on vitronectin transitioned into NutriStem hPSC Medium result in much higher levels of pluripotency with lower levels of spontaneous differentiation (Fig. 2, C) when compared to hPSC cultures maintained on vitronectin in E8.

Key Notes and Take-aways:

To adapt cells to a different medium it is important that the cultures typically are:

- High quality with a high density of cells (≥ 60% confluent).
- Free of differentiating areas. If the cultures contain more than 10% differentiating colonies, the cells should be "cleaned up" by manually removing the differentiated cells.

The type of vitronectin utilized for culture can impact overall culture quality. The NutriStem hPSC Medium cultures presented in the data above were successfully maintained using Vitronectin XF™.

While morphological differences have not been seen to negatively affect the pluripotency of cells cultured in NutriStem hPSC Medium, these differences can be minimized by:

- Supplementing NutriStem hPSC Medium with an additional 100 ng/mL of bFGF.
- Performing a direct adaptation into NutriStem hPSC Medium.

General recommendations:

- Make a frozen stock of the cells in original media prior to adaptation.
- Maintain a set of cell cultures in each of their original media when starting the next level of adaptation as a fallback if the cells do not survive in the next passage.

References:

1. Dakhore, S., et al. 2018. Human Pluripotent Stem Cell Culture: Current Status, Challenges, and Advancement. Stem Cells Int. 2018:7396905.
2. Lanner, F., et al. 2010. The role of FGF/Erk signaling in pluripotent cells. Development. 137: 3351-3360.
3. Greber, B., et al. 2011. FGF signalling inhibits neural induction in human embryonic stem cells. EMBO J. 30: 4874-4884.

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