Transitioning to fully defined HiDef-B8 for adherent human pluripotent stem cell cultures

Introduction

Extensive research has gone into optimizing ideal culture conditions to maintain and expand human pluripotent stem cells (PSCs). Embryonic stem cell (ESC) & induced pluripotent stem cell (iPSC) lines were initially established in media supplemented with animal-derived sera and supported on mouse fibroblasts as a feeder layer. Later, extensive efforts were made to reduce variability such as replacing undefined sera with discrete components including bovine-derived albumin and zebrafish FGF2 (mTeSR™ 1)[1] and pre-coating feeder-free cultureware with extracellular matrix derived from mouse EHS cells (Matrigel®). Notably, many legacy tools are not completely animal-free or defined, are too costly, or simply do not universally work across human PSC lines.[2] A growing shift in the field, and central motivation at Defined Bioscience, has been towards animal product-free systems with fully defined componentry. This led us to commercialize the HiDef-B8 supplement (cat # LSS-204) and HiDef-B8 complete medium kit (cat # LSM-102), a chemically defined, animal-free, serum-free, and reduced-protein PSC growth medium optimized for feeder-free culture systems and weekend-free workflows.

Why Researchers Switch to HiDef-B8

This user guide provides practical approaches to transitioning adherent PSCs to defined, animal-free growth medium that was meticulously optimized for high performance at lower cost. The robustness of HiDef-B8 across >38 PSC lines, pluripotency through >130 passages, and genomic stability through >35 passages is highlighted, under scrutiny of peer-review, in the seminal work by Kuo, *et. al.*[3] (see Figure 1).

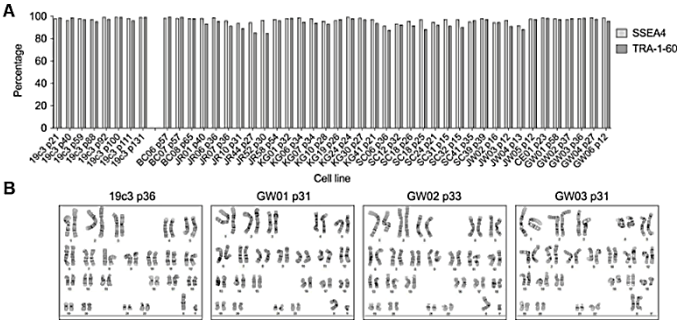
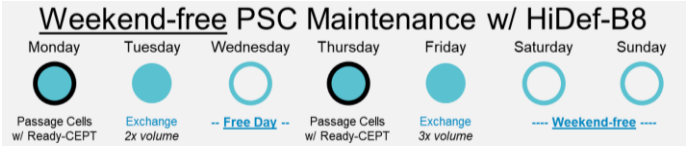


Figure 1A. SSEA4 & TRA-1-60 expression in iPSC lines in B8 across varying passages. B. Karyotype analysis of four iPSC lines derived in B8. (Adapted from Kuo et. al.)[3]

Today, there are several weekend-free solutions to choose from. Our transparency sets HiDef-B8 apart. The fully defined formulation optimized specifically for long-term expansion of human PSCs maintained in a stable, undifferentiated state on a variety of substrates (*e.g.* Matrigel, xeno-free laminins and vitronectin, and animal-origin-free rh-VTN-N) is published[3] and available on request. Knowing the exact componentry of HiDef-B8 will facilitate a straightforward shift from our standard RUO offerings to our cGMP-compliant solutions. For cGMP inquiries, contact info@definedbioscience.com.

HiDef-B8 Advanced Workflows

For PSCs already adapted to HiDef-B8, we recommend twice-weekly gentle aggregate passaging with HiDef-PBS/EDTA (cat # LSR-410) or similar reagent. Example schedule illustrated below:



If enzymatic passaging is performed or cells are plated as singlets, for example, in single-cell clonal expansion or microfluidic sorting, we

suggest using Ready-CEPT[4] cocktail (cat # LSS-301). CEPT is a new best-in-class viability enhancer proven to outperform Y-27632 and ROCK inhibitors for stable, single-cell cloning of human pluripotent stem cells[5]. For gene editing workflows, ask us about custom formulations such as HiDef-B6.

Described below are two tested protocols we recommend for transitioning human PSCs, including iPSCs & ESCs, to HiDef-B8 from mTeSR Plus or other high-protein PSC maintenance media.

Adaptation Protocols

PSCs can be transitioned to HiDef-B8 either **1. directly** or **2. by gradual adaptation**, in active cell cultures or at time of thawing. During adaptation to HiDef-B8, we recommend daily media exchanges and supplementing with Ready-CEPT on day of passage for best results.

1. For direct adaptation, switch media at passage or thaw into 100% HiDef-B8. This approach works best in low-O_2 workflows (2-5%) and with Ready-CEPT.

2. Gradual adaptation involves intermediate steps and an initial 5-day passage (Table 1) prior to adopting our above recommended weekend-free (optional) ~3.5 day passaging schedule.

Table 1. Five-day gradual adaptation to HiDef-B8

Gradual Adaptation Schedule	Day 0	Passage/thaw PSCs from original medium into 75% original + 25% HiDef-B8
	Day 1	Media exchange with 50% original + 50% HiDef-B8
	Day 2	Media exchange with 25% original + 75% HiDef-B8
	Day 3	Media exchange with 100% HiDef-B8
	Day 4-5	Passage with 100% HiDef-B8 and Ready-CEPT
	Day > 5	Passage PSC every 3-4 days in HiDef-B8
	Day > 10-21	Adaptation complete

Best methods for transitioning may vary across PSC lines and may require further optimization or extended passages (P3-P8) to normalize growth rate.

Key Considerations & Observations

The suggested workflows for transitioning to HiDef-B8 involve:

- Adapting and expanding human PSC fastest and most stably under hypoxic conditions (2-5% O_2), but this is not required.
- Using high quality human PSC lines (when available) at ≥60% confluence for best results.
    - “Finicky” or fragile PSC lines may require higher initial seeding density and longer adaptation. We suggest using Ready-CEPT during transition, and recommend karyotyping before and after transition.
- Performing gentle aggregate passage every 3-4 days with HiDef-PBS/EDTA solution or similar non-enzymatic dissociation reagent.
- Pre-coating a substrate suitable for human PSC (*e.g.* Matrigel or AOF rh-VTN-N)

NOTE: Differences in morphology for human PSC cultures between HiDef-B8 and high-protein albumin-rich media such as mTeSR Plus are normal and expected. Cells adapted to HiDef-B8 exhibit a looser morphology and expand in ‘continent-like’ colonies that readily merge, in contrast to large, tight ‘island-like’ colonies with smoother edges typical when using TeSR™ variants. These morphological changes may be mistakenly interpreted as spontaneous differentiation; however, these differences do not negatively affect pluripotency of the cells.

Table 2. Defined Bioscience products mentioned above

Defined Bioscience Reagent	Cat No.
HiDef-B8 400x supplement (1.25 mL)	LSS-204
HiDef-B8 complete kit with DMEM:F12 (500 mL)	LSM-102
Ready-CEPT cocktail, 1000x viability stabilizer	LSS-301
HiDef-PBS/EDTA 1x dissociation buffer	LSR-410

Morphology Comparison

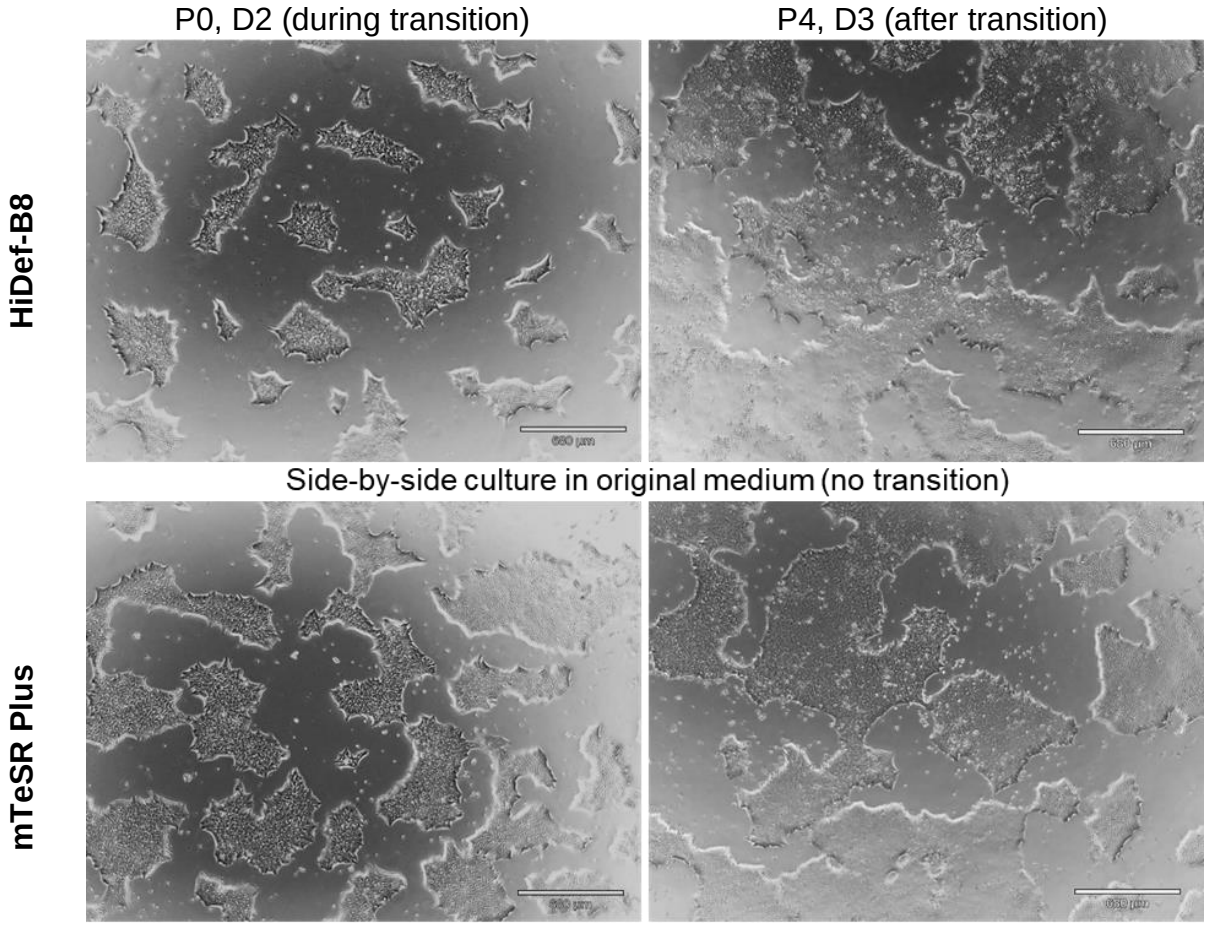


Figure 2 Human PSC cultures transitioned to HiDef-B8 from mTeSR Plus. To maintain the desired confluency, PSCs grown in protein-rich media may require a lower split ratio until fully adapted when transitioning to HiDef-B8.

References

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3. Kuo HH, Gao X, DeKeyser JM, et al. Negligible-Cost and Weekend-Free Chemically Defined Human iPSC Culture. *Stem Cell Reports*. 2020; 14, p256-270. doi:10.1016/j.stemcr.2019.12.007
4. Chen Y, Tristan CA, Chen L, et al. A versatile polypharmacology platform promotes cytoprotection and viability of human pluripotent and differentiated cells. *Nat Methods* 2021; 18, p528–541. doi/10.1038/s41592-021-01126-2
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