User Guide

Getting started with fully defined HiDef® B8 for human pluripotent stem cell adherent 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, inherent variability was addressed by replacing undefined sera with more discrete components, including bovine-derived albumin and zebrafish FGF2 (mTeSR™ 1)[1], and coating cultureware with extracellular matrix derived from mouse EHS cells (Matrigel®). Notably, many legacy tools contain animal-derived or undefined componentry, 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-free systems with fully defined componentry. This led us to exclusively commercialize the fully defined HiDef® B8® Stem Cell Growth Medium (cat # LSS-204 for supplement only; cat # LSM-102 for complete kit), a chemically defined, animal-free, serum-free, and low-protein PSC growth medium optimized for feeder-free culture 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 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 peer-review, in the seminal work by Kuo, *et. al.*[3] (see Figures 1A and 1B).

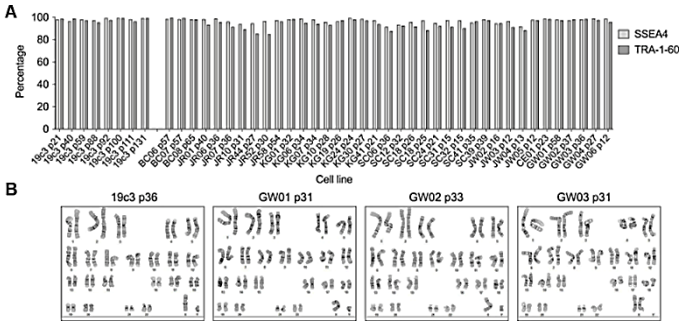
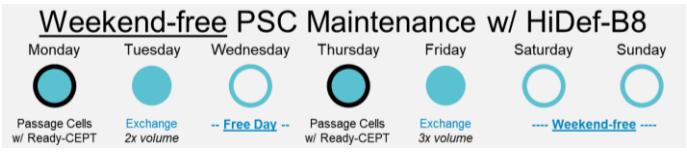


Figure 1A. SSEA4 & TRA-1-60 expression in iPSC lines in B8 across varying passages. B. Karyotype analysis of four iPSC lines derived in B8. (Adapted from Kuo et. al.)[3]

Transparency sets HiDef-B8 apart from other weekend-free solutions. This fully defined formulation, optimized specifically for long-term human PSC expansion, is published[3] (available on request). HiDef-B8 is compatible with a variety of substrates (*e.g.* Matrigel, xeno-free laminins, recombinant laminin fragments, and animal-origin-free vitronectin or rh-VTN-N). Knowing the exact componentry of HiDef-B8 facilitates a straightforward shift from standard RUO offerings to cGMP-compliant and custom solutions. For cGMP and custom inquiries, contact us at info@defined.bio.

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 required or cells are plated as singlets, for example, for microfluidic sorting or single-cell clonal expansion, we

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suggest using Ready-CEPT® (cat # LSS-301). CEPT cocktail is a best-in-class cytoprotectant proven to outperform Y-27632 and other commercial ROCK inhibitors for stable, single-cell cloning of human pluripotent stem cells[4-5].

Described below is a verified protocol we recommend for transitioning human PSCs, including iPSCs & ESCs, to HiDef-B8 from mTeSR Plus or other high-protein content PSC maintenance media.

Adaptation Protocol

PSCs can be switched to HiDef-B8 directly, in active cell cultures at time of passage or at time of thawing. We recommend supplementing with Ready-CEPT during thaw and inoculation, or immediately after passage for best results. Pre-treatment with Ready-CEPT before dissociation is not recommended.

1. **Direct adaptation**: switch media at passage or thaw into 100% HiDef-B8 complete medium supplemented with Ready-CEPT. This approach works best in low-O_2 workflows (3-5%) but this is not required.

2. Gradual adaptation: stepwise and an initial 5-day passage prior to adopting the above recommended weekend-free 3-day/4-day passaging schedule. This approach is not recommended for quick and efficient transition. Contact us for more information.

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:

- **Use high quality human PSC lines** (when available) at ≥70% confluence for best results (Figure 2).

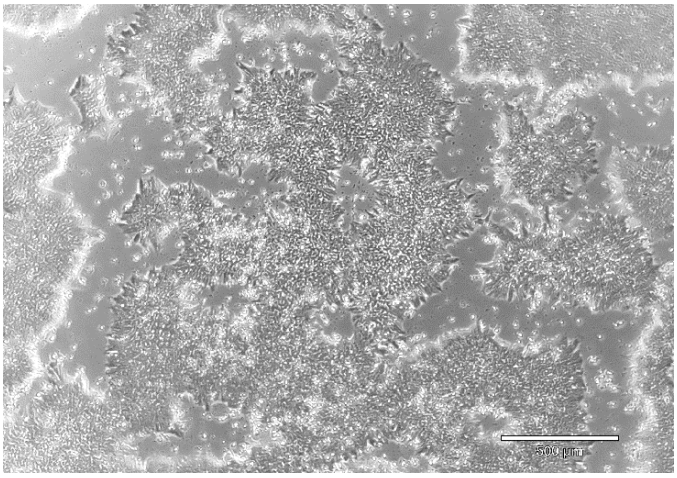

Figure 2. 85% confluent human PSC line 83 in HiDef-B8.

- **"Finicky" or fragile PSC lines may require higher initial seeding density** and longer adaptation.
- **Use CEPT for adaptation to HiDef-B8** over Y-27632, as it more efficiently prevents multiple cellular stress pathways and genetic damage, improving cell quality[5].
- **Routine 3-4 day aggregate passaging helps maintain PSC integrity** using PBS with 0.5 mM EDTA for 3-10 minutes, followed by 4-5 gentle pipette aspirations using complete medium.
    - First rinse with excess PBS/EDTA and remove
    - Add PBS/EDTA to cover colonies for 3-10 min
    - When adherent colonies are appropriately broken up (Figure 3) gently remove PBS/EDTA
    - Suspend aggregates using HiDef-B8 complete medium with Ready-CEPT (Figure 4)
    - Leave adhered cells vs excess manipulation

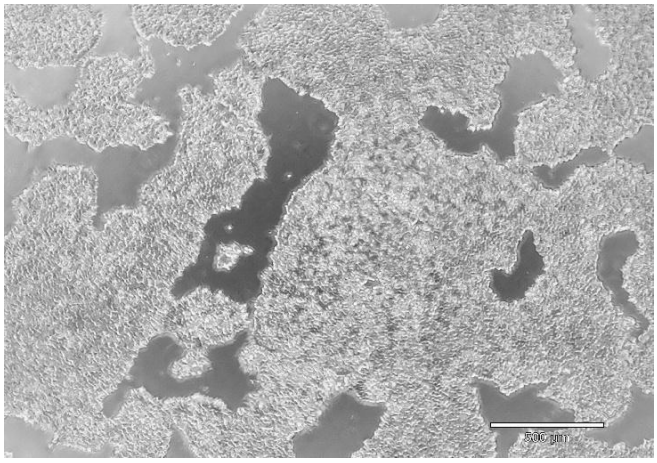

Figure 3. PSC line 83, after 5 minutes in HiDef-PBS/EDTA.

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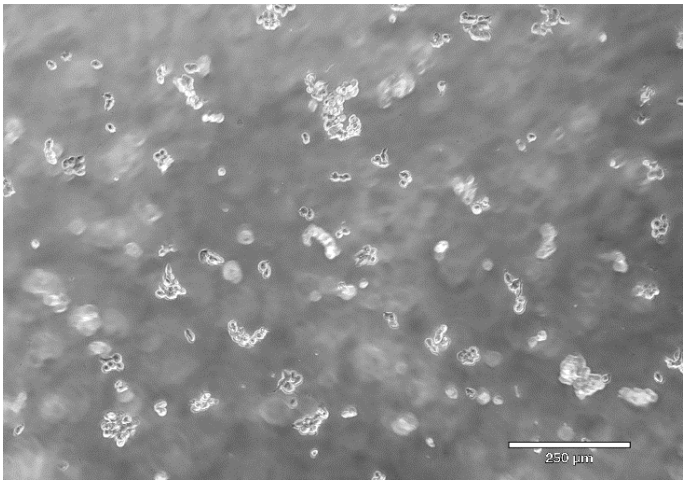

Figure 4: Small aggregate suspension after pipetting four times in HiDef-B8 with Ready-CEPT.

- **B8 is optimized for skip-day workflows** over daily medium exchange. Transition and ongoing maintenance are more successful if you double-feed when skipping one day and triple-feed when skipping two days.

- **Some PSC lines will "dip" or stall at p2-p4** during transition. If this happens, continue exchanging medium following the skip-day, weekend-free maintenance schedule until the cells recover and reach 80% confluence (typically one week). One passage after recovery, PSC are stable, genotypically normal, pluripotent, with a stable growth rate. If it takes longer than a week, or you are not confident cells will recover, please contact us for further discussion at info@defined.bio.

- Adapting and expanding human PSC lines is most rapid and most stable under low-oxygen conditions (3-5% O_2); however, **low oxygen is not required for successful adaptation**.

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, StemFlex™, or StemMACS™ iPS-Brew. These morphological changes may be mistakenly interpreted as spontaneous differentiation; however, these differences do not negatively affect pluripotency of the cells.

Table 1. Defined Bioscience products mentioned above.

Defined Bioscience Reagent	Cat. No.	Format	Primary Use	Recommended Use in This Guide
HiDef® B8® 400x Supplement	LSS-204	1.25 mL supplement; prepares 500 mL complete medium	Fully defined, animal-free human PSC maintenance and expansion medium	Combine with the recommended DMEM/F12 basal medium. Use for transition and routine feeder-free PSC culture.
HiDef® B8® Complete Kit	LSM-102	1.25 mL B8 supplement and 500 mL DMEM/F12	Convenient complete medium kit for human PSC maintenance and expansion	Prepare as directed and use for adaptation, routine feeding, and passaging.
Ready-CEPT®	LSS-301	1,000x viability stabilizer	Improves cell survival and recovery following dissociation, thawing, and stressful manipulations	Add during thawing or immediately after passage, particularly during transition or when plating singlets. Pre-treatment before dissociation is not recommended.
HiDef® PBS/EDTA	LSR-410	1x dissociation buffer	Gentle, enzyme-free aggregate passaging of adherent human PSC cultures	Use for routine 3-4 day aggregate passaging. Incubate approximately 3-10 minutes and gently resuspend colonies in complete medium.

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Morphology Comparison

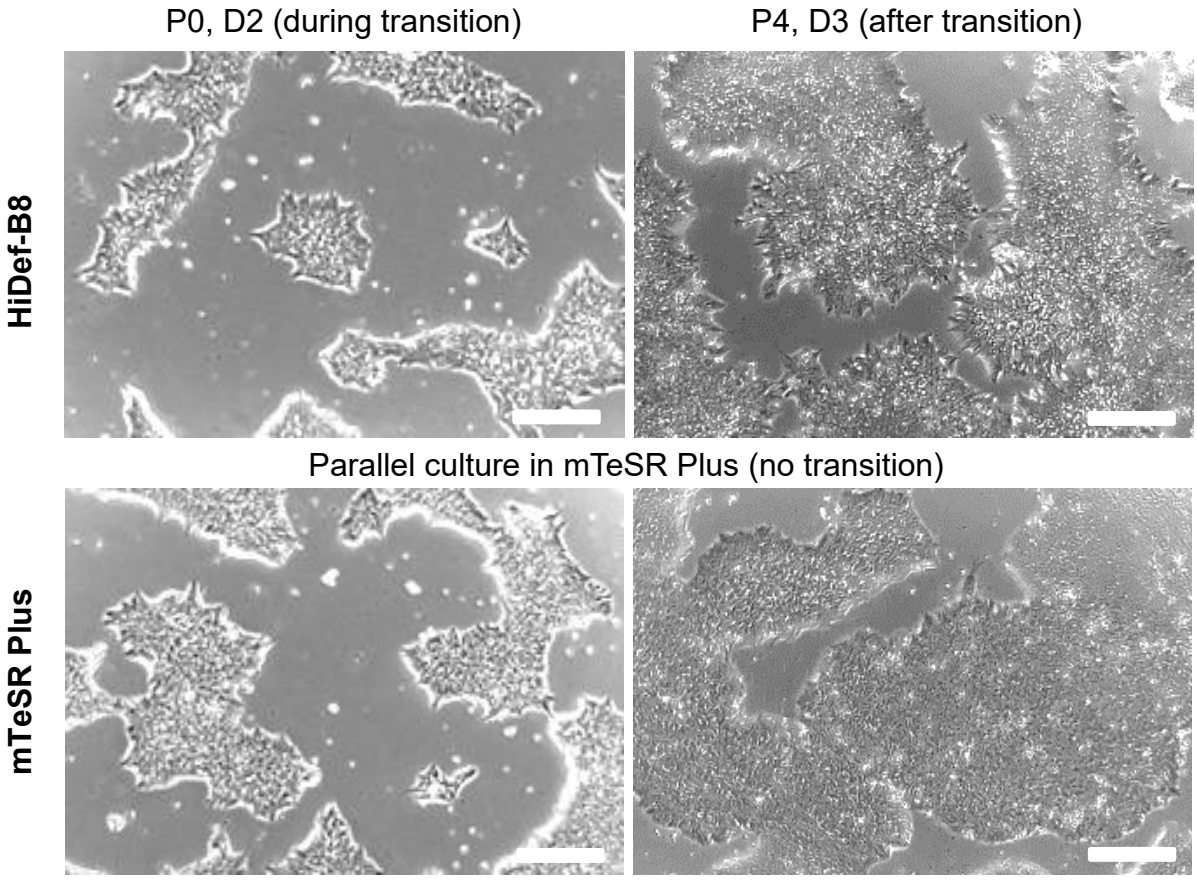

Figure 1. Human PSC cultures transitioned to HiDef-B8 from mTeSR Plus. To maintain the desired confluency, PSCs grown in protein-rich media may require a lower split ratio until fully adapted when transitioning to HiDef-B8. Scale bar = 250 µm

References

1. Ludwig T, Bergendahl V, Levenstein M, et al. Feeder-independent culture of human embryonic stem cells. *Nat Methods* 2006; 3, p637–646. doi.org/10.1038/nmeth902

2. Wesselschmidt RL, Loring JF. Chapter 2 - Human Feeder Cells, Feeder-free, and Defined Culture Systems. *Human Stem Cell Manual* (Academic Press). 2007, p18-33. doi.org/10.1016/B978-012370465-8/50007-7

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.org/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.org/10.1038/s41592-021-01126-2

5. Tristan CA, Hong H, Jethmalani Y, et al. Efficient and safe single-cell cloning of human pluripotent stem cells using the CEPT cocktail. *Nat Protoc* 2023; 18, p58-80. doi.org/10.1038/s41596-022-00753-z

For more information, email us at info@defined.bio, or visit our website at https://definedbioscience.com.

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