HiDef® B8 Stem cell maintenance medium USER GUIDE

Defined, feeder-free maintenance medium supplement for human pluripotent stem cells
Catalog # (supplement only): LSS-204-1, LSS-204-6, LSS-204-12, LSS-204-10M, and LSS-204-C
Catalog # (complete medium): LSM-102-1, LSM-102-6, LSM-102-12, LSM-102-10M, and LSM-102-C

Product Description

Defined Bioscience's HiDef® B8 is a complete, serum-free, fully defined medium for feeder-free maintenance and expansion of human pluripotent stem cells (PSCs) in an undifferentiated state. HiDef-B8 combines robust performance with practical workflow flexibility, supporting weekend-free maintenance and compatibility with a wide range of matrices and passaging methods. The formulation contains insulin, ascorbic acid-2-phosphate, transferrin, sodium selenite, TGFB3, NRG1, and thermostable FGF2/bFGF; no additional components are required.

HiDef-B8 is available in two formats:

1. **Complete medium kit**, including the 400x Supplement and DMEM/F12 basal medium
2. **400x Supplement only** (basal medium not included)

To better support small-scale experiments and pilot studies, the HiDef-B8 400x Supplement is offered in two vial sizes. **Standard vials** prepare 500 mL of complete medium, while **miniature vials** prepare 50 mL, enabling convenient preparation of smaller volumes without excess waste. See instructions below regarding preparation of each format.

Defined Bioscience recommends the use of our DMEM/F12 (catalog # LSB-101) when preparing HiDef-B8 complete medium, as its composition is optimized specifically for HiDef-B8 use. HiDef-B8 400X Supplement compatibility has also been verified with several manufacturers' formulations of DMEM/F12 such as Corning® (#10-092), Gibco™ (#11330), and GenClone™ (#25-503), maintaining human pluripotent stem cells on matrices from several manufacturers including Matrigel® (Corning® #356231), Geltrex™ (Gibco™ #A1569601), and ACS-3035™ Cell Basement Membrane (ATCC®) at coating concentrations as low as 2.4 µg cm^{-2} (e.g. ~1:800 dilution of 18 mg/mL stock matrix at 1 mL per well in 6-well TC-treated plates), in addition to defined substrates such as vitronectin (VTN-N, Gibco™ #A14700). This list of complementary components routinely used in PSC culture is for reference and is not an exhaustive list of verified product compatibility. Contact us at info@definedbioscience.com for more information.

Each lot of HiDef-B8 400X Supplement is used in combination with basal medium in performance testing in a culture assay using human iPSCs.

Contents and Storage

Content	Catalog #	Amount	Storage	Shelf life
Complete Medium Kit				
HiDef® B8 Complete Medium (1 unit)	LSM-102-1	400X Supplement: 1 x 1.25 mL DMEM/F12: 1 x 500 mL	400X Supplement: Store at -20°C protected from light Basal Medium: Store at 2-8°C protected from light	2 years
HiDef® B8 Complete Medium (6 units)	LSM-102-6	400X Supplement: 6 x 1.25 mL DMEM/F12: 6 x 500 mL		
HiDef® B8 Complete Medium (12 units)	LSM-102-12	400X Supplement: 12 x 1.25 mL DMEM/F12: 12 x 500 mL		
HiDef® B8 Complete Medium (Miniature)	LSM-102-10M	400X Supplement: 10 x 0.125 mL DMEM/F12: 1 x 500 mL		
Supplement Only				
HiDef® B8 400X Supplement (1 vial)	LSS-204-1	1 x 1.25 mL	400X Supplement: Store at -20°C protected from light	2 years
HiDef® B8 400X Supplement (6-pack)	LSS-204-6	6 x 1.25 mL		
HiDef® B8 400X Supplement (12-pack)	LSS-204-12	12 x 1.25 mL		
HiDef® B8 400X Supplement (Miniature)	LSS-204-10M	10 x 0.125 mL		

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Preparation of Complete HiDef® B8 Medium (standard vial)

Use appropriate aseptic technique to prepare HiDef-B8 complete medium (DMEM/F12 basal medium + HiDef-B8 400X Supplement). The following example is for preparing 501.25 mL of HiDef-B8 complete medium. If preparing other volumes, adjust accordingly. NOTE: Thaw supplements or complete medium at room temperature (15 - 25°C) and use immediately. Do not thaw in a 37°C water bath, overnight at 4°C, or on ice.

1. Thaw HiDef-B8 400X Supplement and warm to room temperature. Mix thoroughly via inversion or gentle pipetting. It is normal to observe turbidity after thaw. Immediate dissolution should still be observed when added to basal medium (Step 2).
2. Add 1.25 mL of HiDef-B8 400X Supplement (the full volume provided by the manufacturer) to 500 mL of DMEM/F12. Mix thoroughly and avoid foaming.
3. If prepared aseptically, HiDef-B8 complete medium is ready for use. Store complete HiDef-B8 medium at 2 - 8°C for two weeks. Do not freeze or refreeze prepared HiDef-B8 complete medium.

Preparation of Complete HiDef® B8 Medium (miniature vial)

Defined Bioscience now offers HiDef® B8 in a **miniature format**, enabling convenient preparation of small volumes for pilot studies, optimization experiments, and low-throughput workflows. Each miniature 400x Supplement vial is sufficient to prepare 50.125 mL of HiDef-B8 complete medium. Miniature 10-packs collectively yield 500 mL of complete medium, and complete medium kits include a single bottle of DMEM/F12, sufficient for all miniature vials.

1. Thaw the HiDef-B8 400x Supplement and allow it to warm to room temperature. Mix thoroughly by gentle inversion or pipetting. Turbidity may be observed after thaw; this is normal and should fully resolve upon dilution in basal medium (Step 2).
2. Add 0.125 mL of HiDef-B8 400x Supplement (the full vial volume) to 50 mL of DMEM/F12 in a sterile 50-mL conical tube or equivalent vessel. Mix thoroughly, avoiding foaming.
3. When prepared aseptically, HiDef-B8 complete medium is ready for immediate use. Store at 2-8 °C for up to two weeks. Do not freeze or refreeze prepared HiDef-B8 complete medium.

General Pluripotent Stem Cell Adherent Culture Guide

- Use an incubator temperature range of 37 +/- 1°C with humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels. Reduced O_2 tri-gas incubators are encouraged, but not required.
- Split cultures when PSC colonies become too dense, when PSCs show increased differentiation, and/or when colonies cover ~85% of the surface area of the culture vessel, usually every three to five days.
- For standard culture, cells can be passaged at a ratio of up to 1:20 every 4 days after achieving ~70-80% confluence using 0.5 mM EDTA in DPBS (without Ca^{2+} and Mg^{2+}). The split ratio can vary, though it is generally between 1:2 and 1:4 for newly derived PSCs and between 1:3 and 1:20 for established cultures. Occasionally, cells may recover at a different rate and the split ratio will need to be adjusted.
- A general rule is to observe the last split ratio and adjust the ratio according to the appearance of PSC colonies. If the cells look healthy and the colonies have enough space, split using the same ratio. If the colonies are overly dense and crowding, increase the ratio; if they are sparse, decrease the ratio.
- Newly derived PSC lines may contain a fair amount of differentiation through the first 3-5 passages. It is not necessary to remove differentiated material prior to passaging. By propagating/splitting the cells, the overall culture homogeneity should improve throughout the early passages.
- For complete transition to the HiDef-B8 medium, a minimum two-passage adaptation phase is recommended.

Key Characteristics

HiDef-B8 complete medium is a specially formulated defined medium that maintains human pluripotent stem cells in feeder-free and serum-free conditions with reduced hands-on cell culture effort and lower frequency feeding required.

- Feeder-free, serum-free, defined cell culture medium for human PSCs
- Versatile - allows enzyme-free passaging from multiple substrates and supports enzymatic single-cell passaging
- Supports the stable expansion and maintenance of PSC lines for >50 passages without karyotypic abnormalities
- Less frequent cell feeding eliminates the requirement for weekend labor. **Take the weekend off!**

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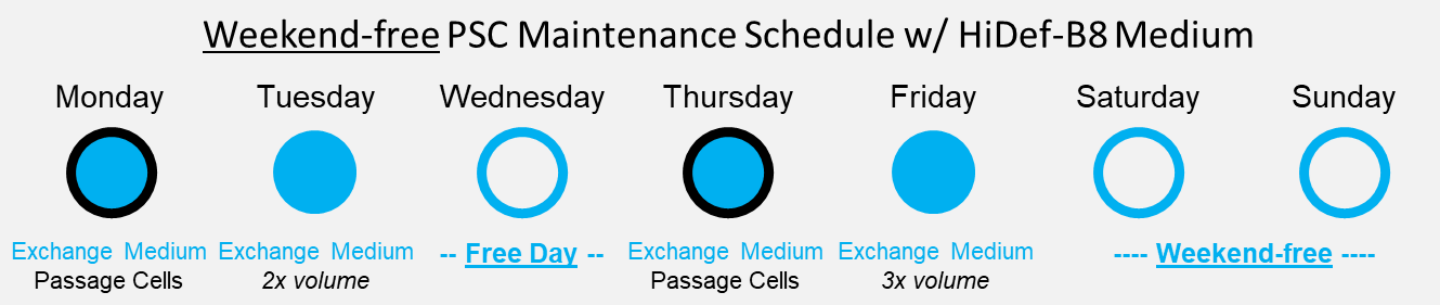

Reference

1. H. H. Kuo, X. Gao, J. M. DeKeyser, K. A. Fetterman, E. A. Pinheiro, C. J. Weddle, H. Fonoudi, M. V. Orman, M. Romero-Tejeda, M. Jouni, M. Blancard, T. Magdy, C. L. Epting, A. L. George Jr., P. W. Burridge, *Stem Cell Rep.* 2020, **14**, 256.

Limited Product Warranty

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