HiDef ™ B8 500X Supplement USER GUIDE

Defined, feeder-free maintenance medium supplement for human iPS cells

Catalog # LSS-201

Product Description

Defined Bioscience's HiDef™ B8 500X Supplement, when added to basal medium, is a complete, serum-free, defined formulation designed for the feeder-free maintenance and expansion of human induced pluripotent stem cells (iPSCs)[1] in the undifferentiated state. The HiDef™ B8 500X Supplement is a unique formulation that offers the modern conveniences of a flexible feeding schedule (including weekend-free maintenance) and also the ability to choose the matrix and passaging reagent that best suits your specific applications. HiDef™ B8 contains insulin, ascorbic acid-2-phosphate (AA2P), transferrin, sodium selenite, thermostable FGF2/bFGF, TGFB3, and NRG1. The addition of further components is not required.

HiDef™ B8 has been validated with DMEM/F12 from Corning® (Corning 10-092-CM) and may be used with either Matrigel (Corning) or Cultrex/Geltrex (Trevigen/Gibco) at concentrations as low as 2.5 µg cm^{-2} (a 1:800 dilution) or higher. Basal DMEM/F12 media and matrices from other manufacturers may also be used once validated in your hands.

Each lot of HiDef™ B8 500X Supplement is used to prepare complete iPSC medium and then performance-tested in a culture assay using human iPSCs.

Contents and Storage

Content	Catalog #	Amount	Storage	Shelf life
HiDef™ B8 500X Supplement	LSS-201	1 x 1mL	Store at -20°C protected from light	1 year

Preparation of Complete HiDef™ B8 Medium

Use sterile techniques to prepare complete HiDef™ B8 medium (DMEM/F12 Basal Medium + HiDef™ B8 500X Supplement). The following example is for preparing 500 mL of 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.

1. Thaw HiDef™ B8 500X Supplement and warm to room temperature. Mix thoroughly. Supplement must be free of cloudiness before adding to basal medium (step 2).
2. Add 1 mL of HiDef™ B8 500X Supplement (the full volume provided by the manufacturer) to 499 mL of DMEM/F12 Basal Medium. Mix thoroughly. If not used immediately, store complete HiDef™ B8 medium at 2 - 8°C for up to 3 weeks. Alternatively, aliquot and store at -20°C for up to 6 months. Do not exceed the shelf life of the individual components. After thawing the aliquoted complete medium, use immediately or store at 2 - 8°C for up to 3 weeks. Do not re-freeze. If prepared aseptically, complete HiDef™ B8 medium is ready for use. If desired, the medium can be filtered using a 0.2 - 0.22 µm low protein binding polyethersulfone (PES) filter unit.

General iPSC Cell Culture Guide

- Use an incubator temperature range of 36°C to 38°C with humidified atmosphere of 5% CO2. Ensure that proper gas exchange is achieved in culture vessels.
- Split cultures when iPSC colonies become too dense, when iPSCs show increased differentiation, and/or when colonies cover ~85% of the surface area of the culture vessel, usually every 3 to 5 days.

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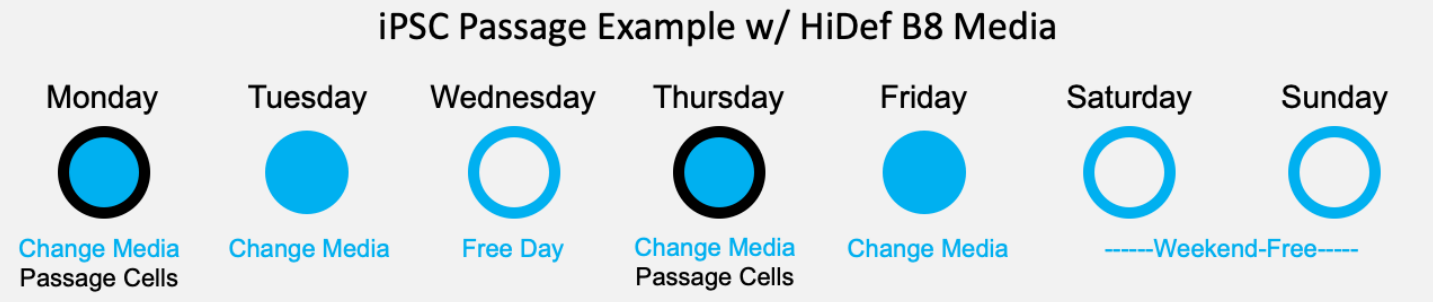
- For standard culture, cells can be passaged at a ratio of up to 1:20 every 4 days after achieving ~70-80% confluence using 0.5 mM EDTA (Invitrogen, 15575020) in DPBS (without Ca2+ and Mg2+, Corning, 21-031-CV). The split ratio can vary, though it is generally between 1:2 and 1:4 for newly derived iPSCs 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 iPSC 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 iPSC 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 health should improve throughout the early passages.
- For complete transition to the HiDef™ B8 medium from other culture systems, a minimum two-passage adaptation phase is recommended.
- HiDef™ B8 has been validated with DMEM/F12 from Corning® (Corning 10-092-CM) and may be used with either Matrigel (Corning) or Cultrex/Geltrex (Trevigen/Gibco) at concentrations as low as 2.5 µg cm^{-2} (a 1:800 dilution) or higher. Basal DMEM/F12 media and matrices from other manufactures may also be used once validated in your hands. Follow manufacturer's recommendations.

Key Characteristics

HiDef B8 Medium is a specially formulated defined media that maintains human pluripotent stem cells in feeder-free and serum-free conditions with less frequent feeding and cell culture time.

- Feeder-free, serum-free, defined cell culture system for human iPS Cells
- Less frequent cell feeding (every other day, weekend free), reducing cost and overall cell culture time
- Supports the culture of pluripotent human iPS cells for greater than 30 passages
- Eliminates the requirement to feed cells over the weekend. **Take the weekend off.**



References

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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