HiDef® S8® Stem cell suspension medium USER GUIDE

Defined, feeder-free medium for high-density expansion of human pluripotent stem cells
Catalog # (supplement only): LSS-604-1, LSS-604-6, LSS-604-12, and LSS-610

Product Description

S8® is a fully defined, serum-free medium within the HiDef® product family, optimized for stirred-tank, microcarrier-free suspension expansion of human pluripotent stem cells (PSCs). HiDef-S8 builds on the performance of Defined Bioscience's existing PSC media by enabling high-yield and scalable expansion workflows, including single-cell or small aggregate inoculation and rapid biomass generation in bioreactors from milliliter to multi-liter scales.

HiDef-S8 supports efficient aggregate formation from single-cell inoculation, enabling consistent growth over multi-day batch runs, and supports expansion to tens of millions of PSCs per mL without the need for microcarriers. PSC aggregates grown in HiDef-S8 maintain high viability, uniform morphology, and stable size distribution throughout culture, supporting efficient expansion under agitation conditions. Following suspension expansion, cells readily reattach in adherent culture and maintain expression of key pluripotency markers over serial passage expansion, supporting both large-scale biomass generation and preservation of PSC identity. Building on the performance of Defined Bioscience's existing maintenance media, HiDef-S8 enables high-yield and scalable workflows, including single-cell or small aggregate inoculation, rapid biomass accumulation, and straightforward downstream transitions for 3D and 2D propagation or differentiation workflows.

HiDef-S8 contains a combination of essential nutrients and growth factors, formulated specifically to maintain pluripotency and support high-density suspension expansion without microcarriers.

HiDef-S8 is currently provided as a 400X Supplement (DMEM/F12 basal medium and optional surfactant NOT included).

To better support small-scale experiments and pilot studies, the HiDef-S8 400x Supplement is offered in two different aliquot formats. 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.

HiDef-S8 is compatible with standard formulations of DMEM/F12 from major suppliers (e.g., Corning®, Gibco®, GenClone®). Optional surfactants include polyvinyl alcohol (PVA, 0.1% w/v), Pluronic F68 (0.1 % w/v), dextran sulfate, and others. Contact us at info@definedbioscience.com for more information.

Each lot of HiDef-S8 400X Supplement is used in combination with basal medium in performance qualification using multiple line PSC suspension cultures under ambient and hypoxic conditions.

S8® is the trademarked name of Defined Bioscience's pluripotent stem cell suspension medium.

Ordering, Contents, and Storage

To purchase HiDef-S8 Stem Cell Suspension Medium, visit our website or email orders@definedbioscience.com. HiDef-S8 400X Supplement is available in 1-, 6-, and 12-unit configurations, and Supplement Mini format is available in 10-packs:

Content	Catalog #	Amount	Storage	Shelf life
HiDef® S8® 400X Supplement (1 vial)	LSS-604-1	1 x 1.25 mL	Store at -70°C protected from light	2 years
HiDef® S8® 400X Supplement (6-pack)	LSS-604-6	6 x 1.25 mL		
HiDef® S8® 400X Supplement (12-pack)	LSS-604-12	12 x 1.25 mL		
HiDef® S8® 400X Supplement (Miniature)	LSS-610	10 x 0.125 mL		

Preparation of Complete HiDef® S8® Medium (standard vial)

Use appropriate aseptic technique to prepare HiDef-S8 complete medium (DMEM/F12 basal medium + HiDef-S8 400X Supplement). The following example is for preparing 501.25 mL of S8 complete medium. If preparing other volumes, adjust accordingly. NOTE: Thaw supplements at **ambient 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-S8 400X Supplement by bringing to ambient temperature. Mix thoroughly via inversion or gentle pipetting. It is normal to observe turbidity after thawing. Immediate dissolution should be observed when added to basal medium (Step 2).
2. Add 1.25 mL of HiDef-S8 400X Supplement (the full volume provided by the manufacturer) to 500 mL of DMEM/F12. Mix thoroughly avoiding foaming.
3. If prepared aseptically, HiDef-S8 complete medium is ready for use. Store complete S8 medium at 2-8°C for up to two weeks. Do not refreeze prepared HiDef-S8 complete medium.

Preparation of Complete HiDef® S8® Medium (miniature vial)

Defined Bioscience also offers HiDef-S8 in a miniature format, enabling convenient preparation of small volumes for pilot studies, optimization experiments, and low-throughput workflows. Each 400x Supplement Mini vial is sufficient to prepare 50.125 mL of HiDef-S8 complete medium. S8 Supplement Mini 10-packs collectively yield 501.25 mL of complete medium.

1. Thaw HiDef-S8 400X Supplement Mini by bringing to ambient temperature. Mix thoroughly via inversion or gentle pipetting. It is normal to observe turbidity after thawing. Immediate dissolution should be observed when added to basal medium (Step 2).
2. Add 0.125 mL of HiDef-S8 400X Supplement Mini (the full volume provided by the manufacturer) to 50 mL of DMEM/F12 in a sterile 50-mL conical tube or equivalent vessel. Mix thoroughly avoiding foaming.
3. If prepared aseptically, HiDef-S8 complete medium is ready for use. Store complete S8 medium at 2-8°C for up to two weeks. Do not refreeze prepared HiDef-S8 complete medium.

General Suspension Culture Guide for Human PSCs

S8 is optimized for scalable expansion of hPSCs in stirred-tank suspension bioreactors with or without perfusion. For an example expansion run in a 1.2-L stirred tank bioreactor system (which demonstrated 60-fold expansion over five days resulting 7 x 10^9 cells), please refer to “High-density pluripotent stem cell expansion in a single-use bioreactor using HiDef S8” on our website, under Resources → App Notes and Posters.

Continuous expansion in stirred tank conditions:

- Temperature: 37 ± 1°C; CO_2: 5%; DO: 40%; pH ~ 7.2
- Agitation: Application-dependent; example ranges include 170-250 rpm (2-L DISTEK BIOne SUB stirred tank with single impeller) and 20-80 rpm (80 mL Eppendorf DASBox Mini or 6-well ultra-low attachment plates). Employ appropriate mixing to maintain viability and ensure homogeneous suspension for your applications. Frequent sampling and measurement may be required while optimizing for each cell line and bioreactor set-up.
- Inoculation density: Application-dependent; example 1-3 × 10^5 cells/mL for single-cell suspension seeding at 95% viability. Increase if cell line shows slower recovery after dissociation. For improved cell viability, include Ready-CEPT® (Defined Bioscience Catalog # LSS-301) during inoculation. 1% polyvinyl alcohol (PVA) or Pluronic F-68 can further support aggregate uniformity and viability where applicable. Maintain viability >85% before inoculation.
- Aggregation: hPSCs in S8 form stable, spherical aggregates suitable for expansion. 100-250 µm is typical for healthy PSC aggregate culture. Decrease agitation for larger aggregates or increase for smaller aggregates. Excessively large aggregates (>400 µm) may lead to necrotic centers and lower expansion yields. Routine visual monitoring is recommended, particularly for unique or novel workflows.
- Harvesting: Collect aggregates by gentle settling or low-speed centrifugation. Avoid high speeds that may compact aggregates and reduce viability. PSC-compatible reagents may be used to return large aggregates to small aggregates or single cells for serial inoculation.
- Re-plating: Aggregates and dissociated cells can transition back to adherent culture, including in HiDef-B8 (Defined Bioscience, Catalog # LSS-204), and exhibit normal morphology within 24 h.
- For bioreactors (e.g. DASBox), use perfusion medium exchange starting with half-volume exchanges 24 hr after inoculation. Increase the rate of exchange each day as cell density increases.

- For culture plates (e.g. 6-well polystyrene), use ultra-low attachment plates with 2-3 mL of S8 medium per well on an orbital rotator or shaker incubator. During medium exchanges, tilt the plate and allow the cell aggregates to settle via gravity or swirl the plates bringing the aggregates to center and aspirate around the edges.

Single-cell passaging: S8 is compatible with enzyme-mediated single-cell passaging approaches commonly used for suspension inoculation. Ready-CEPT may be used at the time of inoculation to facilitate survival after dissociation.

Aggregate passaging: S8 is compatible with EDTA-mediated small aggregate passaging commonly used for suspension inoculation. Ready-CEPT may be used at time of inoculation to facilitate survival after dissociation.

Adaptation from adherent culture: For PSCs transitioning from traditional 2D culture, immediate transition into suspension in S8 is recommended at passage or from thaw, supplemented with Ready-CEPT.

Key Characteristics

S8 complete medium is a specially formulated defined medium that maintains human pluripotent stem cells in feeder-free and serum-free conditions.

- Defined, serum-free, feeder-free medium for high-density human PSC suspension culture
- Supports microcarrier-free, scalable bioreactor workflows
- Enables rapid biomass generation and high viability during multi-passage expansion
- Compatible with single-cell inoculation and Ready-CEPT® supplementation
- Demonstrated maintenance of pluripotency markers and normal morphology, including after re-plating to 2D culture

Limited Product Warranty

Defined Bioscience and/or its affiliate(s) warrant their products as set forth in the Defined Bioscience General Terms and Conditions of Sale. If you have questions, please contact Defined Bioscience at info@definedbioscience.com.