

HFSdaFREE™ KIT (product no. AVB02HFS)

A 3-COMPONENT, CHEMICALLY-DEFINED ANIMAL ORIGIN-FREE (cdAOF) CELL CULTURE SUPPLEMENT SYSTEM FOR HUMAN FIBROBLASTS AND HUMAN HAIR FOLLICLE DERMAL PAPILLA CELLS

 AVANTBIO™ AVANTBIO.COM Phone: +1 206-823-5895	HFSdaFREE™ DEFINED ANIMAL ORIGIN-FREE 5 mls 100X Sterile Concentrate Store at -20°C. Thaw at Room Temp. Avoid Contact or Ingestion. For R&D Use Only. Consult Product Insert For Proper Usage & Handling. PRODUCT AVBP-011 LOT: DOM:	HFGE™ Human Fibroblast Growth Enhancer 0.5 mls 1000X Sterile Concentrate Store Below 8°C For R&D Use Only Avoid Contact or Ingestion Product AVBP-010 LOT: DOM: AVANTBIO™ (206) 823-5895 AVANTBIO.COM	HFGE2™ Human Fibroblast Growth Enhancer 2 1 ml 500X Sterile Concentrate Store at -20°C For R&D Use Only Avoid Contact or Ingestion Product AVBP-040 LOT: DOM: AVANTBIO™ (206) 823-5895 AVANTBIO.COM
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DESCRIPTION AND CONTENTS

- AvantBio Products: HFSdaFREE (product no. AVB02HFS), HFGE (product no. AVBP-010) and HFGE2 (product no. AVBP-040)
- HFSdaFREE: 5 ml Volume, 100X concentrated cdAOF supplement. Sterilized by filtration.
- HFGE: 0.5 ml Volume, 1000X concentrated cdAOF Supplement. Sterilized by filtration.
- HFGE2: 1.0 ml Volume, 500X concentrated cdAOF Supplement. Sterilized by filtration.
- Shipped and stored frozen (-80 °C or -20 °C)
- Shelf life: 18 months from date of manufacture when stored at -20 °C or lower.
- For laboratory usage only. Not to be used for therapeutic or diagnostic purposes in humans or animals. Use good laboratory practices when handling. Avoid contact and do not ingest. Consult SDS.

DIRECTIONS FOR USE

1. To efficiently support the propagation of human fibroblast primary and post-primary cell cultures in the cdAOF environment, AvantBio recommends that HFSdaFREE, HFGE and HFGE2 be used to supplement a **50/50 mixture of EpiLife®** (ThermoFisher Scientific, cat. no. MEPI500CA) and **Human Fibroblast Expansion Basal Medium** (ThermoFisher Scientific, cat. no. M106500).
2. Thaw supplements at room temperature. Use immediately, or store for up to 6 hours at 4 °C before use. Do not leave thawed product at 37 °C, or at room temperature for longer than 20 minutes. Do not re-freeze. Wipe vial clean with 70% isopropyl alcohol before use. HFSdaFREE, HFGE and HFGE2 quantities (volumes) are intended to supplement 500 mls of a 50/50 mixture of EpiLife® / Human Fibroblast Expansion Basal Medium®. For a 500 mL bottle of basal medium, mix each vial throughly and use entire contents of each vial (i.e. rinse each vial 1 x with basal medium).
3. Pre-coating the cell culture surface (substratum) with animal origin-free recombinant human collagen-1 (at 5 ug/cm²), or other animal-sourced collagen-1 (e.g. bovine), can enhance cell adhesion, survival, cell proliferation and replicative lifespan.
4. For both primary culture and post-primary serial propagation, pre-coating the cell culture surface (substratum) with collagen-1 **is required for efficient propagation**. Animal origin free recombinant human collagen-1 can be purchased by request from Collplant Inc. (e.g. Collplant cat. no: W1019-30ml), which is suitable for pre-coating cell culture surfaces.
5. Additionally, the use of Corning® CellBIND® cell culture-ware **may partially replace the requirement for collagen-1 pre-coating** (see: www.corning.com/worldwide/en/products/life-sciences/products/surfaces/cell-bind.html). CellBIND® has been successfully utilized to propagate

post-primary human fibroblasts under cdAOF conditions, but in some cases, at a reduced proliferation rate compared to fibroblasts cultured in the presence of collagen-1 pre-coating. CellBIND® has not been tested on primary human fibroblast cultures.

6. Prior to plating fibroblasts into primary culture, make sure that the cells (cell pellet) are washed essentially free of residual collagenase. Residual collagenase transferred to the primary cell culture can degrade the collagen-1 substratum and inhibit plating of the primary fibroblasts.
7. TrypLE™ Select 1X (ThermoFisher Scientific: cat. no. 12563029) is recommended for animal-free cell dissociation during post-primary serial propagation. For cdAOF cell cryopreservation, it is recommended to use CRYO-free 5 (AvantBio Corp: cat no. AVBP-0102).

AVANTBIO PRODUCT LIMITED USE AGREEMENT

By acceptance of AvantBio's products (e.g. CRYO-free 5™, HKSdaFREE™, HKGE™, HKGE2™, HKSdaFREE2™, HKGE3™, HFSdaFREE™, HFSdaFREE2™, HFGE™, HFGE2™) and any other similar or dissimilar AvantBio products, whether purchased or received free of charge (herein "the transferred products"), the buyer or transferee (herein "the recipient") agrees to the terms of this Limited Use Agreement for the products, as follows:

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