Product Information: StemFit For Differentiation

1. Product Description
StemFit For Differentiation (IDF1) is chemically defined & animal-origin free (CD-AOF) supplement for differentiation of human ES and IPS cells. StemFit For Diff. combined with StemFit medium for the hPSCs expansion can enable clinical applications of hPSC-derived cell lines by providing the CD-AOF culture systems for both hPSCs expansion and differentiation.

StemFit For Diff. is provided as a concentrate and is intended to be used with basal medium (DMEM/F12, RPMI1640, DMEM etc.) and a variety of different induction factors or cytokines.

2. Materials Provided

<table>
<thead>
<tr>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ml</td>
<td>Store at below -20 °C</td>
</tr>
</tbody>
</table>

3. Prepare Differentiation Basic Medium
StemFit For Diff. is provided frozen and should be stored at below -20 °C until use. Use sterile techniques to prepare differentiation medium as follow.

1) Before use, thaw the frozen StemFit For Diff. with occasionally mixing at room temperture (15-25 °C) or overnight at 4°C. CAUTION: Do not thaw StemFit for Diff. at 37 °C as it accelerates the degradation of medium.

2) Add 100 mL of StemFit For Diff. (ESH) to 400 mL basal medium (DMEM/F12, RPMI1640, DMEM etc.) and mix thoroughly to make differentiation basic medium.

Optional: StemFit For Diff. and differentiation basic medium may be aseptically aliquoted and stored at below -20 °C. Once thawed, they may be stored at 2-8 °C for up to 2 weeks (Do not re-freeze). We recommend storing the medium protected from light.

3) To differentiate each lineage, induction factors or cytokines can be added as specified by differentiation protocols.

4) Before use, warm aliquots to room temperature and use immediately.

For Research Use Only, Not Intended For Human or Animal Diagnostic or Therapeutic Uses.
StemFit For Differentiation is a chemically defined, animal component-free supplement for differentiation of human ES and iPS cells to multiple lineages. It can be used with a variety of different induction factors or cytokines to support differentiation along ectoderm, mesoderm, and endoderm lineages.

StemFit Diff, combined with StemFit medium for the human PSCs expansion medium, can enable clinical applications of hPSC-derived cells/tissues by providing the CO-AOF culture systems for both hPSC expansion and differentiation. StemFit Diff, is provided as a 5x concentrate and is intended to be used with basal medium (DMEM/F12, RPMI1640, DMEM etc.) and a variety of different induction factors or cytokines.

Recombinant Proteins for Regenerative Medicine
- Animal-origin free formulation
- GMP compliant product available
- Large batch production
- Frozen form (easy to use)

| Activins A | bFGF |

StemFit For Differentiation can support spontaneous differentiation of hiPSCs via EB formation.

### Method

**Attaching culture (3-5 weeks)**

- **Expansion Medium**
  - StemFit Basic RS (Thermo Fisher Scientific)
  - Growth factor (Thermo Fisher Scientific)
  - 6.3 mm αMEM (Thermo Fisher Scientific)

**EB formation (12 weeks)**

- **Diffusion Medium**
  - DMEM/F12 + StemFit Ent (Stem Cell, Alcobaiba)
  - Combination of DMSO (Thermo Fisher Scientific)

**Analysis of expression by TagIRA™HiPSC Scorpion panel (Thermo Fisher Scientific)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>IPSC</th>
<th>hPSC</th>
<th>ES</th>
<th>EB</th>
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<tbody>
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<tr>
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