

INTRODUCTION

This application will focus on using StemBeads® FGF2 with commercially available defined media for the feeder-free expansion and maintenance of human pluripotent stem cells (hPSCs). StemBeads® FGF2 are microparticles composed of a FDA approved, biodegradable polymer that is loaded with recombinant human fibroblast growth factor 2 (FGF2). StemBeads® FGF2 can be readily adapted to use with a defined pluripotent stem cell media of choice.

PRODUCT HANDLING / DIRECTIONS FOR USE

- Reconstitution & Use:** StemBeads® FGF2 are provided as a ready-to-use 3mL solution in DMEM/F12.
- Storage & Stability:** Upon arrival store at 4°C. StemBeads® FGF2 are stable for up to 6 months without loss of activity when stored at 4°C.
- Release Profile:** 8 µL/mL StemBeads® FGF2 = 10 ng/mL release of soluble FGF2.
- Physical Characteristics:** StemBeads® FGF2 are 15 ± 2 µm in diameter.

For further details concerning StemBeads® FGF2, please refer to StemBeads® FGF2 Product Specification and Information Sheet.

SUGGESTED PROTOCOL

The following protocol describes a procedure that entails splitting once a week and feeding 2-3 times a week for the maintenance and/or expansion of hPSCs. Please note, there may be a slight adjustment period for the first two passages as the cells transition. Additional optimization may be required due to variability between hPSC lines.

Preparation of Media with StemBeads® FGF2

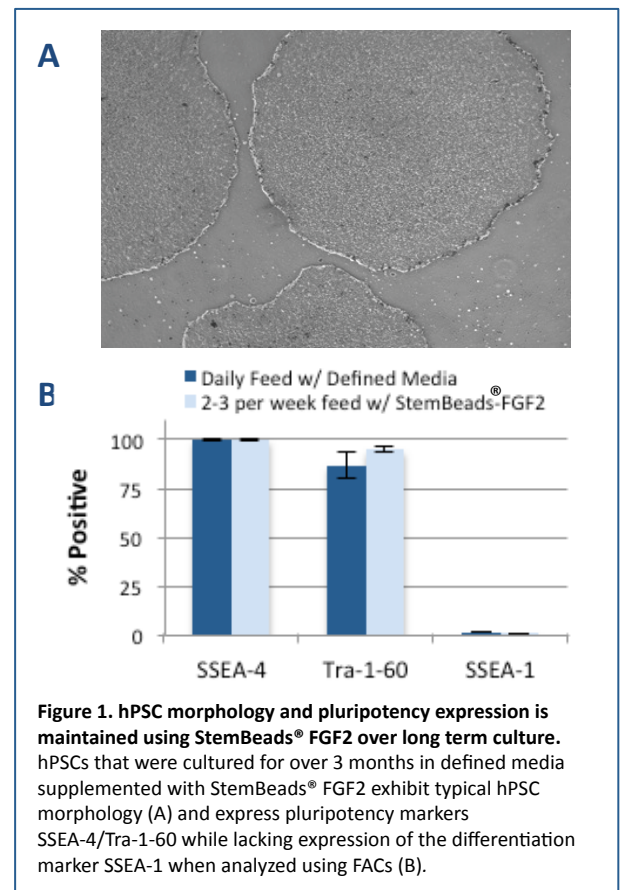
- 1) Aliquot desired volume of defined hPSC media.
- 2) Mix vial of StemBeads® FGF2 thoroughly by vortexing or pipetting prior to use as the beads will settle quickly.
- 3) Add StemBeads® FGF2 into aliquot of defined media at a concentration of 8µL StemBeads® FGF2 per 1mL of media for a 10ng/ml release.

Culturing hPSCs with StemBeads® FGF2

- Day -3:** Remove media and replace with freshly prepared StemBeads® FGF2 supplemented media.
- Day 0:** Dissociate and split hPSCs using preferred enzymatic treatment, then re-plate the hPSCs in defined media supplemented with StemBeads® FGF2 onto a pre-coated vessel.
- Day 1 (Optional):** If a large amount of unattached/dead cells are observed, wash 2x with DMEM/F12* and replace with freshly prepared StemBeads® FGF2 supplemented media.
- Day 4:** Remove media, wash 2x with DMEM/F12* and replace with freshly prepared StemBeads® FGF2 supplemented media.
- Day 7:** Repeat splitting and feeding as described above.

*Note: Washing is highly recommended prior to every feed to remove cell debris and excess beads.

DATA



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