

### SUGGESTED PROTOCOL: Feeder-free Maintenance & Expansion of hiPSCs/hESCs using StemBeads® FGF2

This protocol uses StemBeads® FGF2, feeding only 2-3 times a week, and splitting cells once a week to effectively maintain/expand human pluripotent stem cells (hPSCs). It was developed using multiple lines of human induced pluripotent stem cells (iPSCs) and human embryonic stem cells (hESCs). Some optimization may be required due to variability between hPSC lines. StemBeads® FGF2 can be used in whichever FGF2-dependent hPSC culture medium you normally use.

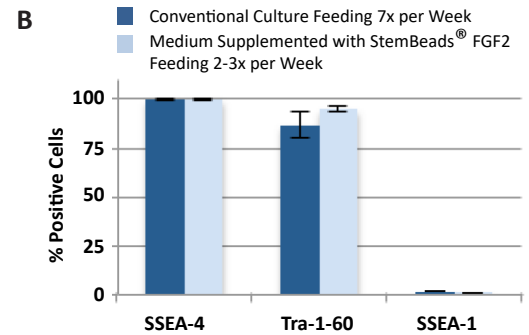
#### Preparation of Media with StemBeads® FGF2

- 1) Mix vial of StemBeads® FGF2 thoroughly by vortexing or pipetting prior to use.
- 2) Add 8 µl of StemBeads® FGF2 per 1 mL of medium. This will provide cells with stable 10 ng/mL FGF2.

#### Culturing hPSCs with StemBeads® FGF2

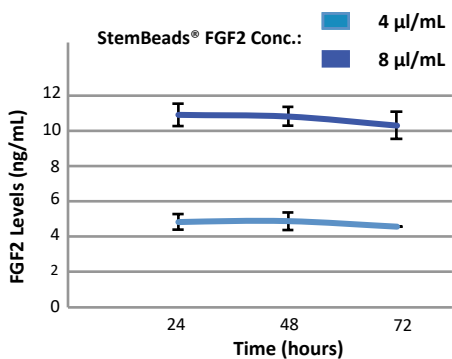
- Day -3: Remove medium from cultures and replace with StemBeads® FGF2 supplemented medium.
- Day 0: Split hPSCs using preferred enzymatic method, then plate into pre-coated culture dish in medium supplemented with StemBeads® FGF2.
- Day 1 (optional): Wash 2x with medium e.g. DMEM/F12 if large amount of unattached cells are observed and replace with StemBeads® FGF2 supplemented medium.
- Day 4: Remove culture medium, \*wash 2x with medium e.g. DMEM/F12 and replace with StemBeads® FGF2 supplemented medium.
- Day 7: Repeat split and feed as described above, Day 0 - Day 4.

\*Note: Washing is highly recommended prior to each feed to remove cell debris and remaining beads.

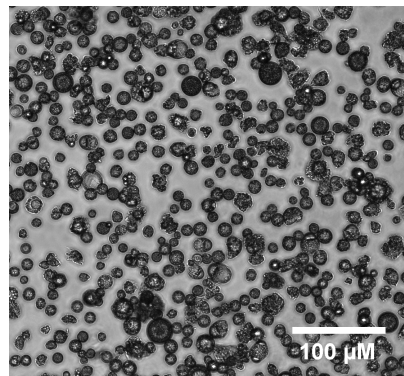


**Figure 1. Long term growth of hPSCs in StemBeads® FGF2 maintains pluripotency.** hPSCs were cultured over 3 months in medium supplemented with StemBeads® FGF2. A) Cells maintain typical hPSC colony morphology and B) express pluripotency markers SSEA4/Tra-1-60 and lack the differentiation marker SSEA-1, quantified by FACS.

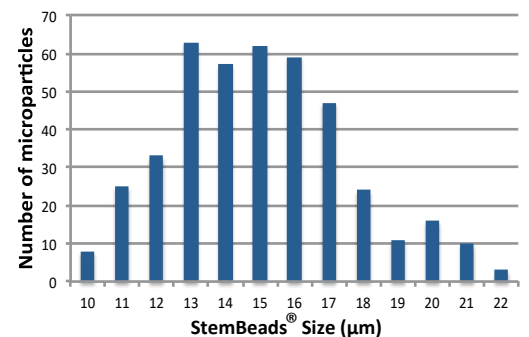
#### A StemBeads® FGF2 Release Profile



#### B



#### C StemBeads® FGF2 Size Distribution



**Figure 2. StemBeads® FGF2 Sustained Release Profile and Standard Size Distribution.** A) StemBeads® FGF2 maintain a stable FGF2 concentration in hPSC cultures over 3 days. 4 µL/mL or 8 µL/mL StemBeads® FGF2 generate a stable release of 5 ng/mL or 10 ng/mL FGF2 protein respectively. B) Phase image of StemBeads® FGF2 under 32X objective. C) Size distribution of StemBeads® FGF2; Analysis performed using ImageJ (NIH).