CORE BIOPROCESS

FGF-2 TOP®

(*Thermostability Optimized*)

FGF-2 TOP® a novel, thermostabilized growth factor that allows you to grow FGF-2-dependent cell cultures more efficiently with fewer media changes.

About FGF-2 TOP® (*Thermostability Optimized*)

Through novel protein engineering, FGF-2 TOP® offers an increased half-life and therefore fewer feedings are required compared to the wild-type growth factor. It is significantly more tolerant of heat while maintaining full bioactivity, leading to improved homogeneity in your cell cultures.

Some applications that benefit from FGF-2 TOP® are:

Pluripotent Stem Cells

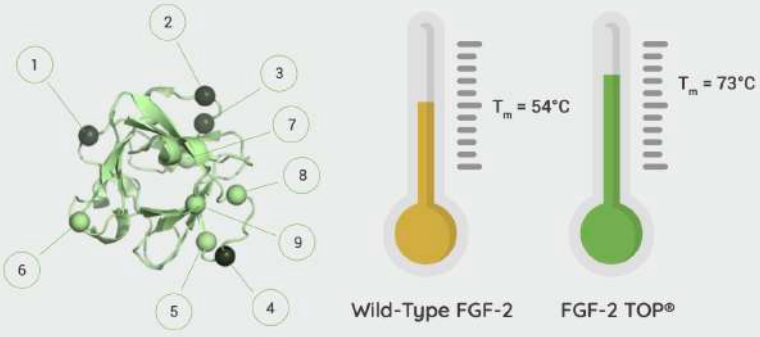
Organoids

Neural Stem Cells

A Convenient Alternative With Cost Advantages

In order to maintain pluripotency and avoid spontaneous differentiation of PSCs, scientists traditionally had to maintain a strict daily feeding schedule due to the short half-life (approximately 9 hours at 37°C) and temperature sensitivity of wild-type FGF-2.

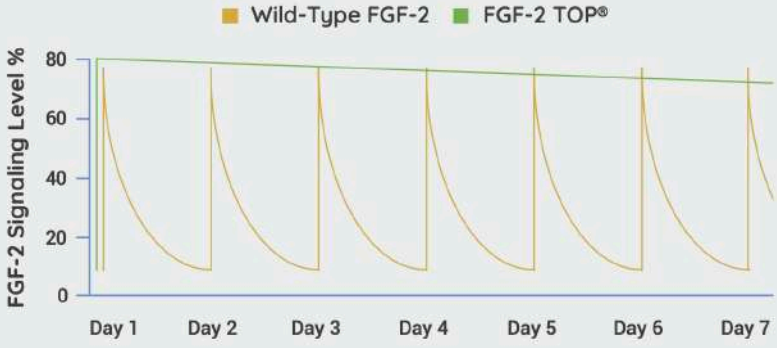
Improvements in Half-Life at 37°C



To improve stability, a novel nine amino acid substitution of the wild-type FGF-2 was performed, improving the heat stability of FGF-2 TOP® resulting in an increase in half-life under cell culture conditions at 37°C, from 10hrs (wild-type FGF-2) to > 7 days (FGF-2 TOP®).

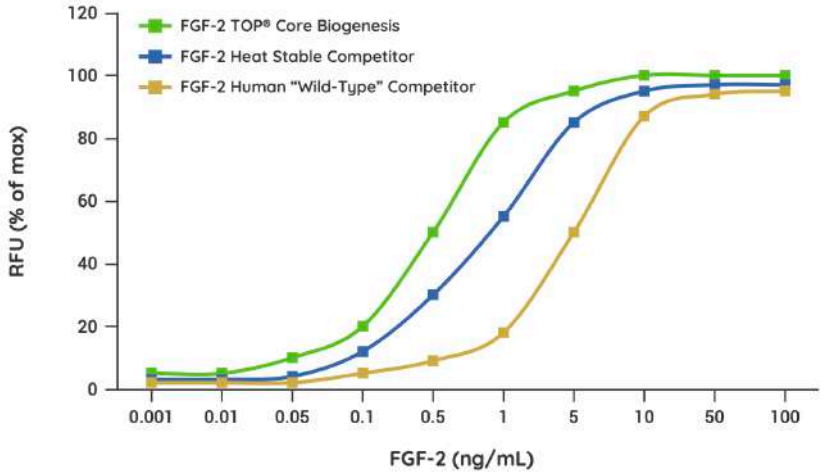
Supplement Less and Achieve More Consistency

By nature of its increased half-life and stability, FGF-2 TOP® presents a constant exposure of growth factor (green line) in contrast to the short half-life and signaling of the wild-type protein that must be replenished daily (yellow line). Thus, feeding schedules are more streamlined and cell culture phenotype is more homogenous.



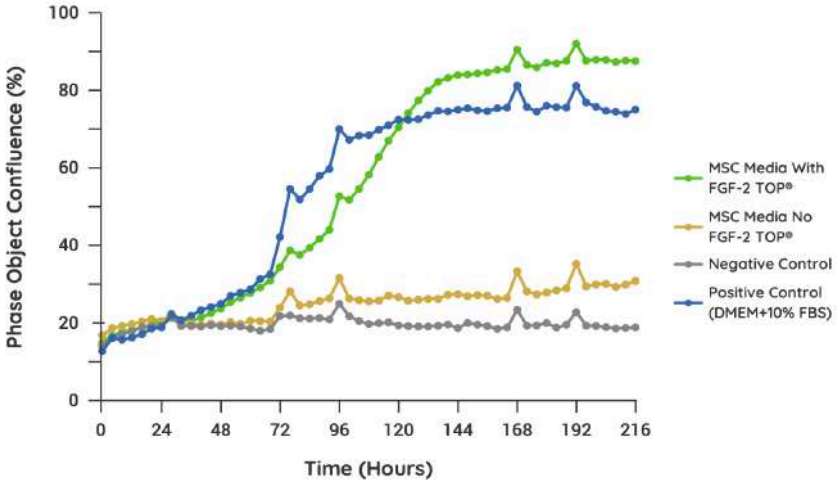
The Stability to Support Your Development and Scale-Up

Bioactivity Preserved, Improved Cell Response



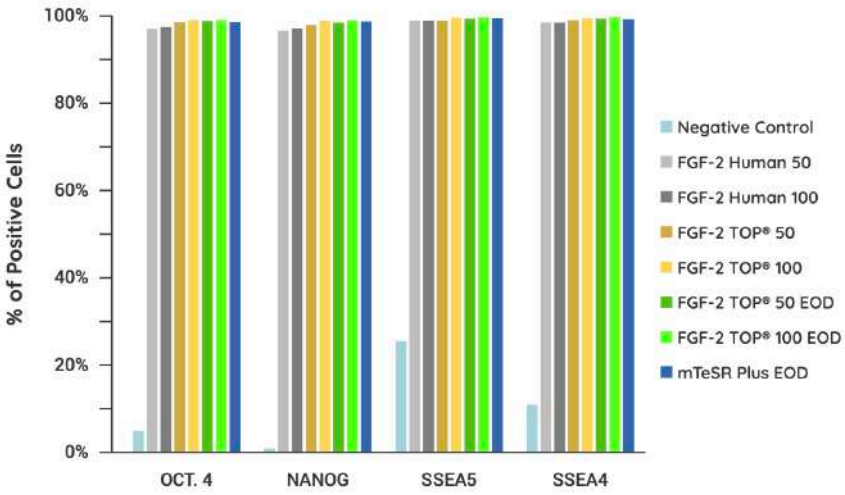
Comparison of FGF-2 TOP® with a FGF-2 heat stable competitor and human FGF-2 wild-type in a 3T3 cell proliferation assay using varying concentrations of each protein after a 48-hour incubation at 37°C. FGF-2 TOP® demonstrates maintenance of full bioactivity and with a 5-fold lower EC50 -FGF-2 TOP® demonstrates a greater capacity to promote 3T3 cell proliferation and at lower concentrations than competing heat stable alternatives and wild-type FGF-2 in this model.

Supporting Proliferation of Adherent Cell Culture



In an internal MSC model analyzing confluence over 9 days, the addition of FGF-2 TOP® to an internal MSC formulation resulted in an equivalent cell confluence and expansion (as assessed through cell attachment) compared to the positive control containing 10% FBS. Similar results have been observed in both iPSC and adherent HEK-293 models.

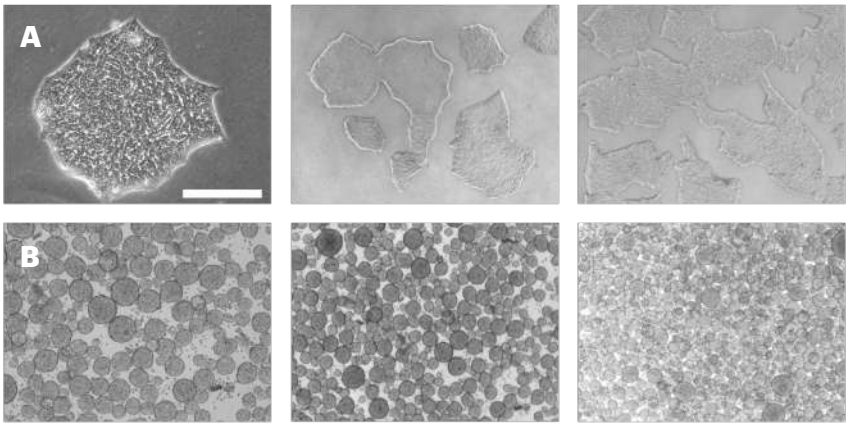
Maintenance of Pluripotency and Stemness

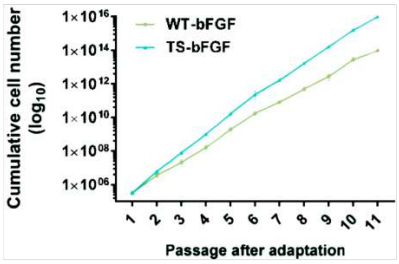


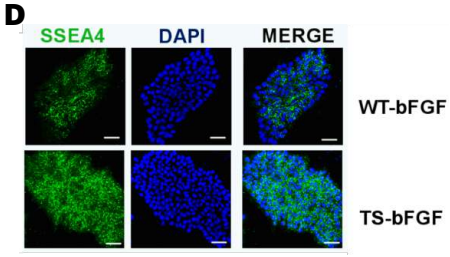
FGF-2 TOP® maintains high-quality iPSC cultures under various concentrations and media feeding regimes as assessed by flow cytometry. Cells were treated with wild-type "human" FGF-2 or FGF-2 TOP® at 50 or 100 ng/mL either daily if not indicated or every other day (EOD). The negative control contained no FGF-2 supplementation. All tested conditions resulted in high expression of pluripotency markers. Most notably, EOD feeding with FGF-2 TOP® showed equivalent levels as well as provided further proof of bioactivity maintenance after stability changes.

The Performance to Optimize Your PSC Cultures & Differentiation

Intensified iPSCs expansion & marker expression

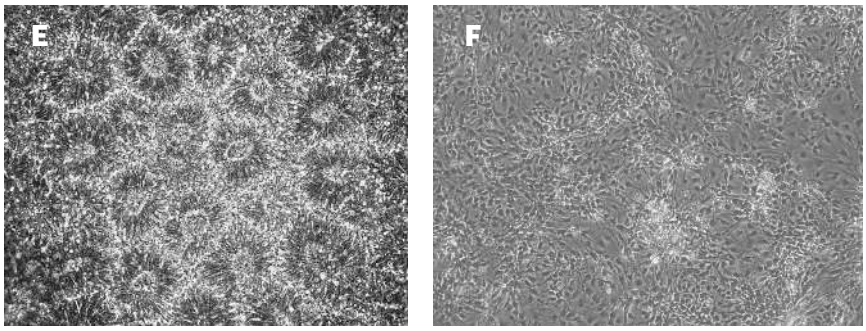






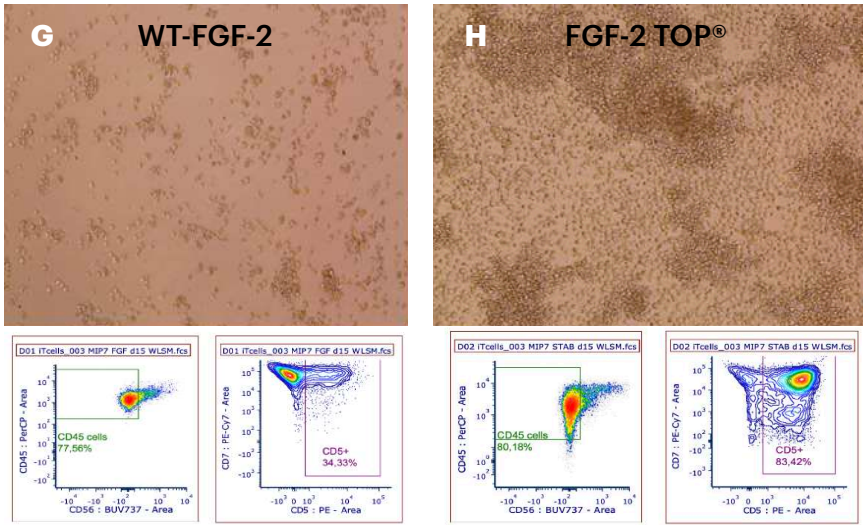
FGF-2 TOP® provides superior performance for high-quality iPSC cultures. A) When iPSCs are cultured in adherent conditions (2D) and supplemented with 100ng/mL of FGF-2 TOP®, cells display typical colony-forming morphologies, preventing the appearance of differentiated cells. B) When iPSCs are expanded in suspension (3D) and supplemented with 100ng/mL of FGF-2 TOP®, cells form embryoid bodies (EBs) with consistent shape and size. C, D) iPSCs cultured during several passages (>10) result in higher cell numbers and increased expression of pluripotency markers, when media is supplemented with Thermostable FGF-2 TOP® compared to Wild-Type FGF-2.

Robust differentiation into NSC phenotypes



FGF-2 TOP® supports differentiation of iPSCs into Neural Stem Cells. E) Neural rosettes are formed from dissociated EBs when supplemented with 20ng/mL of FGF-2 TOP®. F) Cells transferred from the neural rosettes into pO/Lam-coated plates, start to differentiate into Neural Progenitor Cells (NPCs) reaching 80-90% of confluence after culture with 20ng/mL of FGF-2 TOP®.

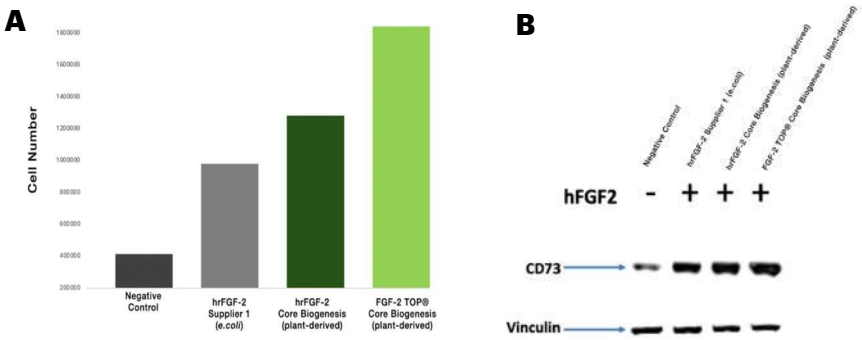
Improved development of iT-Cells

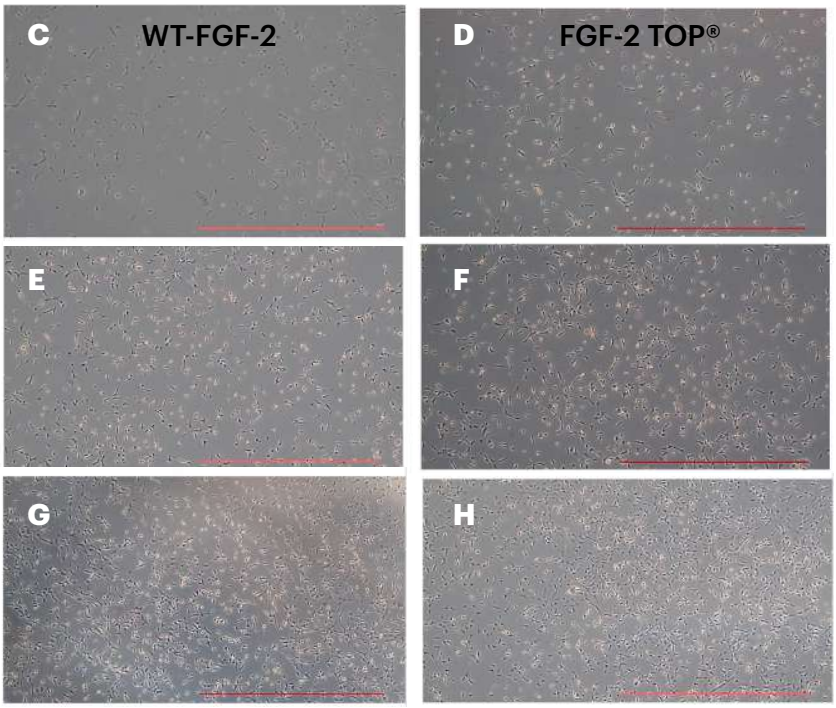


FGF-2 TOP® enhances the generation of Hematopoietic Stem Progenitor Cells (HSPCs) for the development of iT-cells. G, H) iPSCs were differentiated into HSPCs following EB formation protocol, employing 50ng/mL of either Wild-Type FGF-2 (G) or FGF-2 TOP® (H). Results indicate FGF-2 TOP® yields higher cell numbers, and iT-cells development capacity.

The Consistency for Your Results while Working with MSCs Cultures

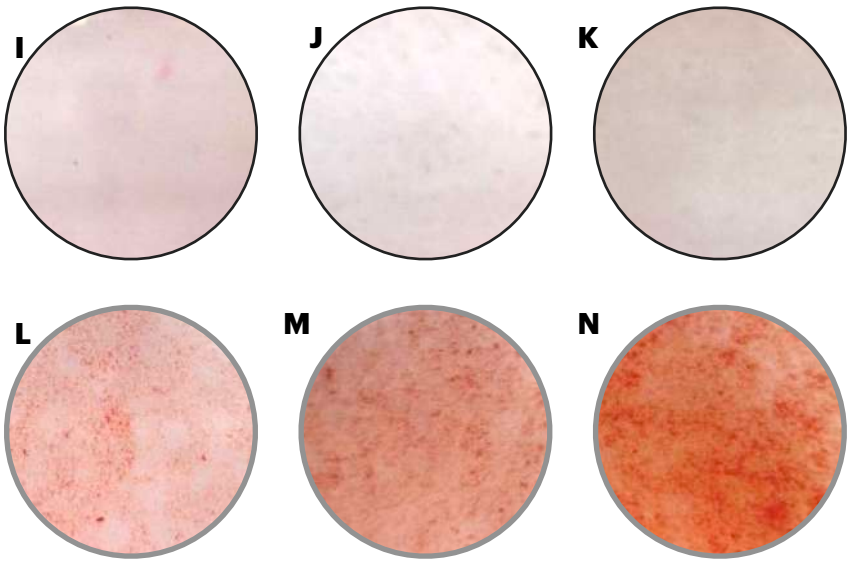
Increased cell numbers during MSC maintenance





Exogenous supplementation FGF-2 TOP® promotes higher increase of cell proliferation rates than "Wild-Type" FGF-2 on hMSCs. A) After 7 days in culture, hMSCs cultured with the wild-type version of hrFGF-2 from 2 conditions (Supplier 1, *e.coli* derived - reference standard), and (Core Biogenesis, plant-derived) promoted a cell expansion from 2x10^5 (seeding density) to 9.81x10^5 (Supplier 1) and to 12.83x10^5 (Core Biogenesis). Furthermore, when hMSCs were supplemented with FGF-2 TOP® (Core Biogenesis) cell expanded from the same seeding density to 18.42x10^5. B) Western Blot analysis revealed CD73 (-) cell phenotype when cultured without FGF-2 supplement (negative control), while all conditions containing FGF-2 in the media formulation were CD73+, showing the highest marker expression in cultures with FGF-2 TOP®. C,E,G) hMSCs cultured at days 3 (C), 5 (E), and 7 (G) with 10ng/mL of wild-type FGF-2. D,F,H) hMSCs cultured at days 3 (D), 5 (F), and 7 (H) with 10ng/mL of FGF-2 TOP® achieve higher confluency than the non-thermostable growth factor.

Boosted Differentiation Capacity



hMSCs cultured with FGF-2 TOP® provide superior differentiation capacity into the osteogenic linage. I-N) After culturing hMSCs for 7 days with various conditions of FGF-2 supplementation: I,J,K: no FGF-2 supplement; L,M: 10ng/mL supplement of wild-type FGF-2 (L, supplier 1) and (M, Core Biogenesis); N: 10ng/mL of FGF-2 TOP®, cells underwent differentiation employing a cocktail for osteogenic induction during 12 days. Alizarin red analysis revealed higher coloration and differentiation degree into osteoblastic cells from hMSCs cultured under FGF-2 TOP® supplementation conditions.

Recombinant FGF-2 TOP® (*Thermostability Optimized*)

RUO I GMP

FGF-2 TOP® recombinant protein is derived from a proprietary, plant-based production system that ensures high purity and sustainability. Completely animal component-free, it is a safer and more ethically sourced choice for stem cell research and cell therapy applications. Our unique bioproduction process delivers significant cost savings. Additionally, by eliminating the need for repeated supplementation and daily medium changes, expenses can be further reduced and convenience enhanced, streamlining workflows for greater efficiency. With our ultra-scalable manufacturing method and ability for proteins to be stored in the seeds, supply chain bottlenecks are eliminated, ensuring a reliable source of both RUO and GMP-compliant grade recombinant proteins.

Why Choose FGF-2 TOP® ?

- High purity >95-97%
- 100% animal-component free
- Improved cell response EC50<0.5 ng/mL
- Optimized cell culture homogeneity
- Weekend free iPSC feedings
- Sustainable
- Ultra-scalable supply capacity
- 100% preservation of bioactivity
- Significant cost savings at small and bulk needs
- Thermostability saves time and money

EXAMPLE iPSC FEEDING SCHEDULE

Available for Research Use + cGMP Processes

FGF-2 TOP® (*Thermostability Optimized*) Specifications:

	FGF-2 TOP® RUO	FGF-2 TOP® GMP-Compliant
Amino Acid Sequence	154 amino acids with 9 aa point mutations from the wild-type FGF-2. Tag-free. Original reference sequence accession number: P09038	154 amino acids with 9 aa point mutations from the wild-type FGF-2. Tag-free. Original reference sequence accession number: P09038
Origin	Plant-derived (*Camelina sativa*)	Plant-derived (*Camelina sativa*)
Identity	Molecular weight via SDS-PAGE	Molecular weight via SDS-PAGE, HPLC
Bioactivity	EC50	EC50, IUs
Purity	> 95%	> 95-97%
Concentration	1mg/mL (Lyophilized)	1mg/mL (Liquid in PBS)
Mycoplasma	No	PCR
Bioburden	< 10 CFU/mL	USP <61>
Endotoxin	< 100 EU/mL	USP <85> ≤ 50 EU/mL
Shelf Life	24 months	Stability studies in progress
Release	COT	COA
Shipping Conditions	Room temperature	Dry ice
Storage Temperature	-20ºC / -80ºC	-80ºC

Nucleus Biologics

FGF-2 TOP® in cGMP-Compliant Grade is manufactured and released in collaboration with Nucleus Biologics (San Diego, CA).

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