

TCB Transition Guide

Product Code(s)	HB12632
	HB11050
	HB17550
Product Name	TCB-32
	TCB-541
	TCB-621
Purpose	Maintenance of pluripotent stem cells.

Please note: These products are for RESEARCH USE ONLY and are not intended for therapeutic or diagnostic use. Not for human or veterinary use



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1. Products Overview

Traditional cell culture protocols rely on recombinant proteins like FGF2 to maintain pluripotency and drive proliferation. However, these proteins are characterized by high thermal instability, often losing the majority of their biological activity within 24 hours at 37°C. This decay necessitates daily media changes, creating an unstable signaling environment of extreme peaks and troughs that does not mimic physiological conditions. The TCB Series (TCB-32, TCB-541, and TCB-621) represents a shift toward small-molecule FGFR1 agonists. These compounds are chemically defined, lean molecules ($\approx 900\text{Da}$) that target the same FGF signaling pathways as their protein counterparts but with total thermal stability. The TCB series of molecules have a number of key advantages compared to FGF2:

- **Total Thermal Stability:** TCB molecules maintain 100% biological activity for at least 13 days in culture media (37°C), eliminating the need for daily media replenishment.
- **Steady-State Signaling:** By providing a constant signal rather than daily spikes, TCB agonists create a more predictable and reproducible environment for delicate expansion and differentiation protocols.
- **Equivalent Biological Exposure:** Quantitative modeling of cumulative signaling (Total Exposure) demonstrates that single dose TCB agonists provide a biological signal statistically equivalent to FGF2.
- **Sustained Pluripotency and Expansion:** Literature-derived data confirms that TCB-32 supports the continuous expansion of cell cultures over 30+ days and multiple passages while maintaining confluency and health markers.
- **High-Potency Dose-Response:** The TCB series exhibits a classic sigmoid dose-response profile, allowing researchers to operate at a Saturation Plateau ($2\mu\text{M}$ for TCB-32) for maximum signaling reliability.

Unlike "heat-stable" recombinant proteins (e.g., FGF-G3) which merely extend the decay window, the TCB series utilizes a completely different chemical class to achieve superior stability at physiological temperatures. This makes them an ideal tool for long-term automated culture, organoid development, and large-scale expansion where media handling must be minimized.

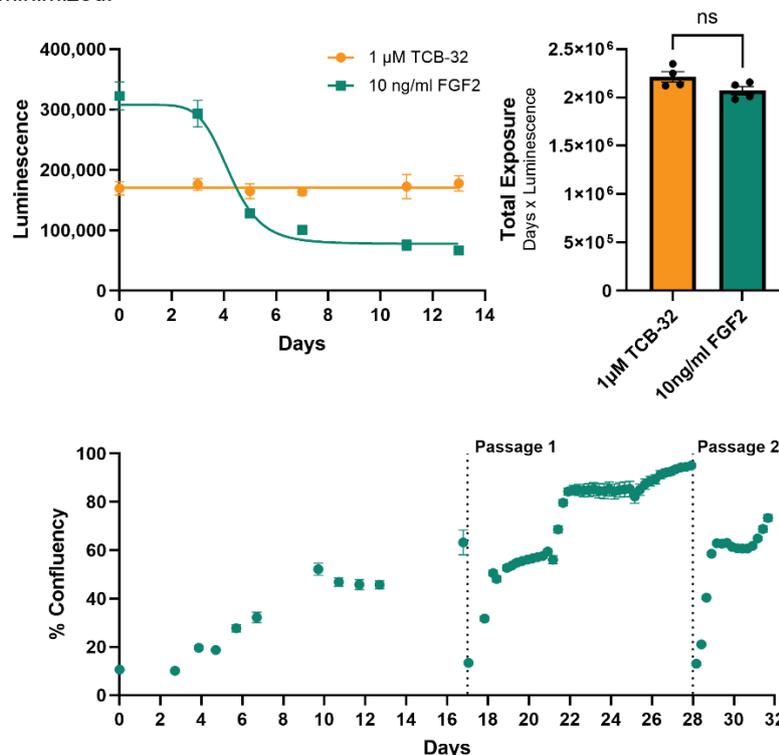


Figure 1. Superior stability of TCB-32 in cell culture media compared to FGF2. A, B. TCB-32 is stable for at least 13 days in cell culture media and provides equivalent total FGF2 exposure to 10ng/ml FGF2. **C** TCB-32 maintains C2C12 cells for at least 3 passages in serum-free media.

2. Handling and Storage

TCB molecules are significantly more stable and easier to handle in the laboratory environment than recombinant FGF2 products:

- Shipping and Long-Term storage:** TCB-32, TCB-541 and TCB-621 are shipped at ambient temperature and should be stored at -20°C upon arrival for up to 1 year.
- Stock Stability:** Solutions of TCB-32 can be stored at +4°C for up to 1 month or for 6 months at -20°C
- Reconstitution:** To prepare a stock solution of TCB-32, TCB-541 and TCB-621 (10mM in DMSO) we recommend using our [Molarity Calculator](#) to calculate the volume of anhydrous DMSO to add then vortexing thoroughly. Prior to use we recommend filter sterilising through a DMSO compatible 0.2µm sterile filter. We do not recommend attempting to use aqueous stocks of TCB compounds due to limited solubility in dH₂O above working concentrations.

For ease of transition, we have prepared a matrix of equivalent pack sizes between FGF2 and TCB-32, TCB-541 and TCB-621 using recommended working concentrations.

FGF2 Pack Size [#]	TCB-32 equivalent	TCB-541 equivalent	TCB-621 equivalent
20µg	2mg	1mg	500µg
50µg	-	2mg	1mg
100µg	10mg	-	2mg
250µg	25mg	10mg	-
500µg	50mg	-	10mg
1000µg	100mg	50mg	25mg

Table 1. Approximate equivalent yield of supplemented media between FGF2 and TCB-32, TCB-541 and TCB-641. [#]Calculated assuming a working concentration of 20ng/ml FGF2, 2µM TCB-32, 1µM TCB-541 and 500nM TCB-621.

3. Application Guide

The optimal protocol for TCB usage to replace FGF2 will vary between cell line and experimental system. We suggest these guidelines are used as a starting point for internal optimization and validation within your experimental system. Comparisons between FGF2 and TCB molecules are provided in Table 2 and Figure 2.

1. Add 10mM TCB stock solution (in DMSO) to base media at the recommended concentration shown in Table 2. Ensure that the stock is added dropwise to the media while swirling to avoid precipitation.
2. Culture cells in the prepared media at 37°C.
3. Monitor cells daily for health, pluripotency and signs of metabolic stress. While TCB molecules are stable for up to 14 days in cell culture conditions, it is likely that the media will be ready to be changed before this point (e.g. nutrient depletion, pH shift). Conversely, literature indicates that TCB-32 supports consistent expansion and confluency over 30+ days and multiple passages when maintained correctly.

	FGF2	TCB-32	TCB-541	TCB-621
Recommended Stock Concentration	100µg/ml	10mM		
Solvent	PBS + 5% Trehalose	DMSO		
Recommended Working Concentration	20ng/ml	2µM	1µM	500nM
Maximum Working Concentration*	100ng/ml	2µM	1.4µM	3µM
Saturation Plateau	30ng/ml	2µM	500nM	300nM
EC50	14.5ng/ml	0.71µM	0.43µM	0.11µM
Final DMSO Concentration	NA	0.2%	0.1%	0.05%
Molecular Weight	≈18,000	892.9	927.3	916.9
Thermal Stability (37°C)	Up to 3 days (FGF2)	100% Activity for up to 13 days		
Recommended Media Change	Daily	Every 7 – 14 days		

Table 2. Equivalence matrix between FGF2, TCB-32, TCB-541 and TCB-621. *TCB molecule concentrations are limited by solubility in aqueous media, not toxicity. Toxicity is not observed with TCB-32 until concentrations in excess of 30µM.

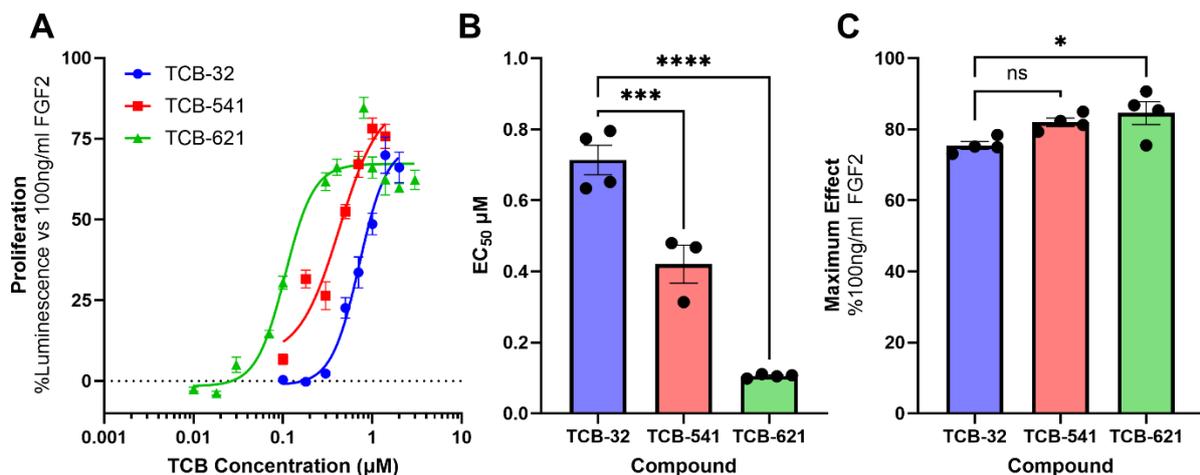


Table 2. Pharmacodynamic comparison of TCB-32, TCB-541 and TCB-621. **A** Dose response curves of luminescent cell growth assay normalized to 100ng/ml FGF2-G3 growth curve. **B and C.** Calculated EC₅₀ and maximum biological effect normalized to 100ng/ml FGF2 of the three molecules respectively.

4. Guidelines, precautions, troubleshooting

Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the assay kit.

Problem	Potential Cause
Sudden growth drop	Above the recommended maximum working concentration listed in Table 2 TCB compounds can precipitate which then inhibits growth. We recommend sticking to the working concentration suggested in Table 2.
Media yellowing before 14 days	While TCB-32 maintains 100% biological activity for 14 days, cells continue to consume nutrients and produce lactic acid. In high-density cultures, media changes should be performed based on pH indicators (Phenol Red shift) to manage metabolic health, even though the growth factor signal remains stable.
Precipitation upon addition to media	Pre-warm the media and add dropwise while swirling to avoid precipitation of the TCB compound. Ensure not to exceed the recommended concentrations in Table 2.
Variable growth rates between different cell lines at the same TCB dose.	Different lines (e.g., iPSCs vs. Organoids) may have varying densities of FGF receptors. While 2 μ M is the saturation point for many systems, some lines may require minor titration between 0.1 μ M and 2 μ M to match the kinetics of your previous protein-based protocol.
DMSO stock solution appears "frozen" or solid at room temperature	High-purity DMSO can solidify in a standard air-conditioned lab. Ensure the 10mM master stock is fully thawed and vortexed before use to ensure accurate volumetric pipetting.

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