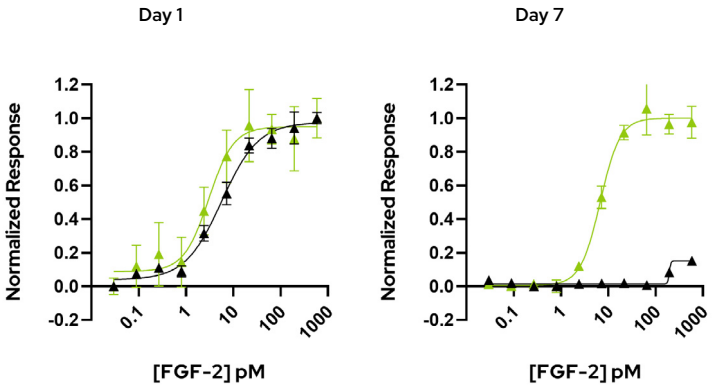
Technote

Thermostable FGF2-G3 protein (Qk053) remains stable and highly bioactive



Quantitative luciferase reporter assay showing FGF2-G3 (154 aa) (Qk053, green) and WT FGF-2 (154 aa) (Qk027, black) both have comparable bioactivity at day 0 (EC50 = 3 pM and 5.7 pM respectively) but only FGF2-G3 remains bioactive at day 7 (EC50 = 6.8 pM) compared to WT FGF-2 (EC50 = 195.6 pM).

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Introduction:

Fibroblast growth factor 2 (FGF-2) is an essential growth factor for regulating several biological processes and a critical component for stem cell maintenance in feeder-free media. Qkine FGF2-G3 (Qk053) was engineered with nine amino acid substitutions to stabilize its structure and expressed in E. coli creating the only animal-free, protein-tag-free stabilized FGF2-G3 available commercially. The enhanced stability makes FGF2-G3 a highly bioactive and thermostable (heat stable) engineered form of FGF-2, resulting in improved culture conditions with less frequent media changes.

Methods:

FGF2-G3 (Qk053) and WT FGF-2 (Qk027) bioactivity were compared and determined using the Promega serum response element luciferase reporter assay in transfected HEK293T cells. Cells were treated in triplicate with a serial dilution of FGF2-G3 or WT FGF-2 for 3 hours. Firefly luciferase activity was measured at days 0 and 7 and normalized to the control Renilla luciferase activity.

Results:

FGF2-G3 (Qk053) and WT FGF-2 (Qk027) have equivalent bioactivity at day 0. FGF2-G3 maintained stable bioactivity after 7 days when compared to WT FGF-2 which had a significant reduction in bioactivity. FGF2-G3 provides a reliable source of animal-free, highly bioactive, and thermostable FGF-2 for stem cell culture and FGF2-dependent applications. The use of stable FGF2-G3 results in enhanced stem cell cultures with less frequent media changes improving self-renewal maintenance and enhancing the reproducibility of subsequent differentiation.the reproducibility of subsequent differentiation regenerative medicine applications.