Qkine

3D Bio-Tissues
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Application note

CytoBoost™-enhanced growth factor activity for cell culture and bioprocessing

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Introduction

The cellular environment is crowded with macromolecules, unlike typical dilute cell cultures. This crowding affects the behavior of growth factors and enzymes. Despite its many potential benefits, macromolecular crowding is not widely used *in vitro*. CytoBoost™ was developed as a versatile, easy-to-use macromolecular crowding additive compatible with different cell types, culture settings, and bioprocessing applications.

In this collaborative application note CytoBoost™ formulations, Perform and Maximise are tested for their impact on the bioactivity of Qkine high quality growth factors TGF-β1 PLUS™ (Qk010), TGF-β3 (Qk054), bovine/porcine FGF-2 (Qk056), IGF-1 (Qk047), EGF (Qk011) and thermostable FGF-2, FGF2-G3 (Qk053).

Background

The intra- and intercellular environment is vastly different from the dilute solutions typically used to grow and maintain cells in culture. A key distinction lies in macromolecular crowding, a phenomenon where the cytoplasm and interstitial fluid are densely packed with macromolecules like proteins, polysaccharides, nucleic acids, lipid vesicles, etc, all contributing to create volume exclusion effects. Macromolecular crowding significantly influences protein behavior, including that of crucial growth factors and enzymes. Despite the many potential benefits, the use of macromolecular crowding in vitro is far from being a widespread feature. This may be due to unreliable outcomes resulting from poorly characterized culture conditions, or a lack of obvious cost-efficiency gain, including higher cell production yields, shorter culture times, and lower media costs. To address this gap, 3D Bio-Tissues have developed CytoBoost™ (Figure 1), a new range of macromolecular crowding additives that are versatile in many cell culture and bioprocessing applications, easy to use in both 2D and 3D culture settings, and broadly compatible with primary, secondary, immortalized, and induced pluripotent stem cells.

Figure 1:
The CytoBoost™ Perform and Maximise: two versatile, ready-to-use additives from our premium cell care range for *in vitro* culture systems.

Illustrative Case Studies

3D Bio-Tissues and Qkine collaborated to test the effect of two of the most versatile CytoBoost™ formulations, Perform and Maximise (Figure 1), on the bioactivity of various growth factors commonly used in cell culture. Qkine growth factors are widely recognized as having high lot-to-lot consistency in bioactivity, high purity, and being produced in animal origin-free systems.

Methods

Bioactivity was determined using a quantitative firefly luciferase reporter assay in HEK293T cells (TGF-β1 PLUS, TGF-β3, pFGF-2, FGF2-G3, EGF) or MCF-7 cells (IGF-1). Cells maintained with DMEM or Opti-MEM culture media were treated in triplicate with a dose response of each growth factor. CytoBoost™ Perform or CytoBoost™

Maximise were added to treated wells at a final 4% (v/v) concentration. Equivalent volumes of PBS were added to control wells. Firefly luciferase activity was measured and normalized to control Renilla luciferase activity to calculate growth factor kinetics.

Results

CytoBoost™ formulations Perform and Maximise have previously been shown to promote muscle and fat cell proliferation (see application note). To determine the effect of the CytoBoost™ formulations on the acute bioactivity of individual growth factors and cytokines, we performed quantitative luciferase reporter assays with and without the addition of CytoBoost™ formulations.

The addition of Perform increased the maximal response of TGF-β1 PLUS (Figure 2) and TGF-β3 (Figure 3), however no significant change on EC50 was observed for these growth factors. A similar response was observed for bioactivity assays with porcine FGF-2, FGF2-G3 (Figure 4), or EGF (Figure 5), where a trend of higher maximum activity and similar of slightly lower EC50 was observed for all growth factors.

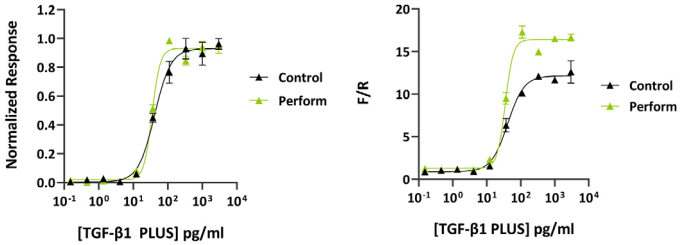

Figure 2:
Effect of Cytoboost™ Perform on TGF-β1 PLUS activity in a CAGA-response element luciferase reporter assay using transfected HEK293 cells (6 h). EC50 TGF-β1 = 41.4 pg/ml, TGF-β1 + Perform = 35.1 pg/ml.

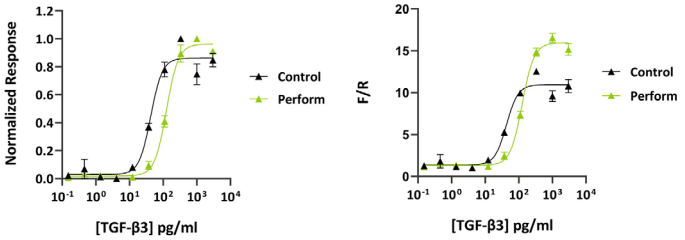

Figure 3:
Effect of Cytoboost™ Perform on TGF-β3 activity in a CAGA-response element luciferase reporter assay using transfected HEK293 cells (6 h). EC50 TGF-β3 = 42.8 pg/ml, TGF-β1 + Perform = 125.6 pg/ml.

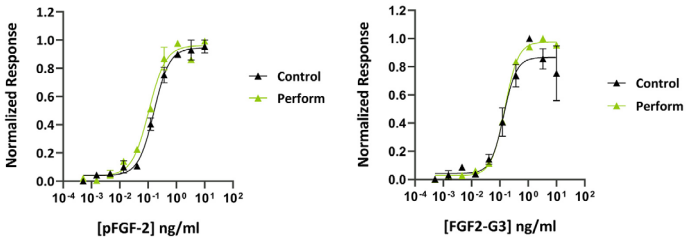

Figure 4:
Effect of Cytoboost™ Perform on porcine FGF-2 (left) and thermostable FGF-2 (FGF2-G3) activity (right panel) in a serum response element luciferase reporter assay using transfected HEK293 cells (3 h). EC50 pFGF-2 = 0.16 ng/ml, pFGF-2 + Perform = 0.11 ng/ml; EC50 FGF2-G3 = 0.14, FGF2-G3 + Perform = 0.15 ng/ml.

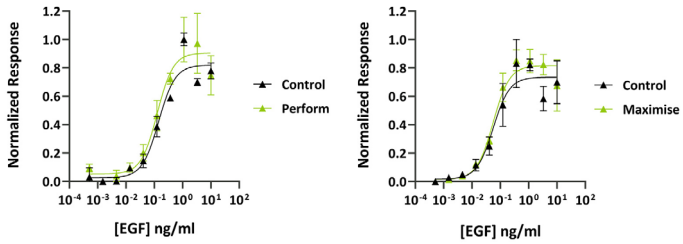

Figure 5:
Effect of Cytoboost™ Perform (left) and Maximise (right panel) on EGF activity in a serum response element luciferase reporter assay using transfected HEK293 cells (3 h). EC50 EGF = 0.14 ng/ml, EGF + Perform = 0.13 ng/ml. EC50 EGF = 0.06 ng/ml, EGF + Maximise = 0.05 ng/ml.

Conversely, Perform promoted the bioactivity of IGF-1 by slightly lowering its EC50 but without affecting its maximal response (Figure 6). CytoBoost™ Maximise had comparable effects, with a slightly increased maximum activity of EGF (Figure 5) and decreased EC50 of TGF- β1 PLUS (Figure 7). Although the impacts on growth factor bioactivity observed in the luciferase assays were modest and not statistically significant, this may be due to the short incubation time (3-6 h). Greater effects of the CytoBoost™ formulations may be observed on longer term cell proliferation.

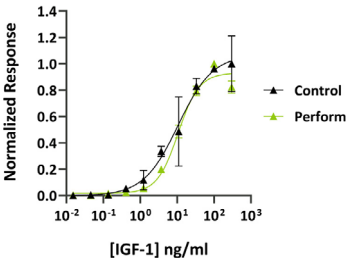

Figure 6:
Effect of Cytoboost™ Perform on IGF-1 activity in a serum response element luciferase reporter assay using transfected MCF-7 cells (4 h). EC50 IGF-1 = 10.4 ng/ml, IGF-1 + Perform = 9.8 ng/ml.

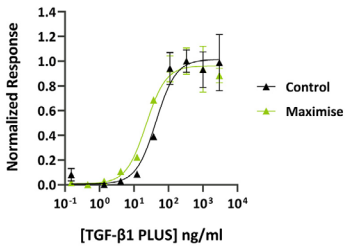

Figure 7:
Effect of Cytoboost™ Maximise on TGF-β1 activity in a CAGA-response element luciferase reporter assay with HEK293 cells (6 h). EC50 TGF-β1 = 43.6 ng/ml, TGF-β1 + Maximise = 22.4 ng/ml.

Conclusions

By mimicking crowding *in vitro*, CytoBoost™ offers a promising avenue for enhancing growth factor bioactivity and improving cell culture and bioprocessing outcomes.

How Macromolecular Crowding Influences Growth Factor Activity

Macromolecular crowding impacts the bioavailability and kinetics of biomolecules in multifaceted ways:

Excluded Volume Effects: by increasing the effective concentration of soluble molecules and promoting intermolecular interactions. This can enhance ligand-receptor binding and stabilize protein conformations, potentially increasing growth factor binding and signaling kinetics.

Diffusion: The higher density of crowded environments hinders diffusion, potentially slowing growth factor degradation and prolonging their activity.

Protein-Protein Interactions: Crowding favors protein complex formation, which can either enhance (e.g., receptor dimerization) or inhibit (e.g., aggregation) growth factor activity.

Conformation and Stability: Crowding can change the viscosity and electrostatic interactions of a liquid environment, and shift protein conformational equilibria towards more compact and stable states, potentially enhancing growth factor stability and activity. Please see 3D Bio-Tissues for more details.

Other potential applications of CytoBoost™ in cell culture and bioprocessing

Stem Cell Expansion and Differentiation: Crowding could be leveraged to control stem cell fate and direct their differentiation into specific cell types.

Protein Production and Stability: Crowding could enhance protein production and stability in bioreactors, improving biopharmaceutical manufacturing.

Media Optimization: Crowding could be exploited to reduce the concentration of essential growth factors in media, including animal sera and albumins, thus reducing costs and addressing ethical and sustainability concerns.

Biosensing and Drug Discovery: Crowding can make biosensors and cellular and tissue models of disease more sensitive and accurate by better reproducing the original biological systems.

Key Message:

Macromolecular crowding is a fundamental aspect of cellular environments both in vivo and in vitro. Scan the QR code or visit www.3dbiotissues.com to learn more.

For more information

Please contact our team: **customerservice@qkine.com** if you would like to discuss commercial or academic collaborations, supply agreements or any aspects of growth factor optimization and other products.

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