Potent Cell Signaling and Sustained Proliferation with OptiKine EGF, Liquid Recombinant Animal-Free EGF

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APPLICATION BENEFITS

- ***Ready-to-use sterile liquid rhEGF that requires no reconstitution***
- ***Supports robust Vero cell expansion in a defined, serum-free system***
- ***Potency equivalent to a leading GMP rhEGF product***
- ***Manufactured free from serum, microbial fermentation, and mammalian cells***
- ***Minimizes endotoxin and host cell protein contaminants***
- ***Supports vaccine, biomanufacturing, and epithelial cell culture workflows***

INVITRIA SOLUTIONS

OptiKine EGF - Recombinant human epidermal growth factor

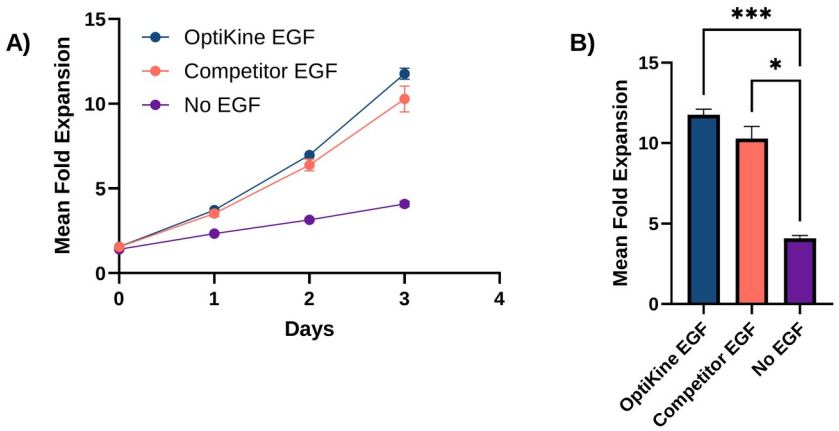
OptiVERO - Serum-free medium for Vero cell culture

EXECUTIVE SUMMARY

OptiKine EGF, InVitria's sterile, ready-to-use recombinant human epidermal growth factor (rhEGF) solution, is a high-purity, GMP-grade growth factor manufactured in an animal-free system free from the limitations of microbial fermentation, and adventitious agent risk of mammalian cells. In this study, OptiKine EGF was evaluated for bioactivity and its ability to support Vero cell expansion in a defined, serum-free system. OptiKine EGF activated EGFR-driven ERK phosphorylation, exhibited potency equivalent to a leading competitor EGF in a BALB/3T3 proliferation assay, and drove robust, EGF-dependent Vero expansion comparable to competitor products. Together, these results establish InVitria OptiKine EGF as a sterile, animal-origin-free, like-kind replacement suitable for biomanufacturing and cell culture workflows without the need for reconstitution.

Key Highlights

- InVitria's high-purity, GMP-grade, recombinant human epidermal growth factor (rhEGF) enables robust expansion of Vero cells
- Matches the performance of leading GMP rhEGF products, enabling a seamless like-kind replacement in a liquid format
- Manufactured in a system free from serum, microbial fermentation, and mammalian cells, minimizing endotoxins and host cell protein contaminants



OptiKine EGF supported Vero cell expansion comparable to competitor EGF.

INTRODUCTION

Epidermal growth factor (EGF) is a critical supplement for the in vitro expansion of numerous epithelial and adherent cell types, including epidermal keratinocytes (Boyce & Ham, 1983), corneal and limbal epithelial cells, intestinal stem cells and epithelial organoids (Sato et al., 2009), and Vero cells (Litwin, 1992), where it provides a chemically defined alternative to serum-derived growth factors and is commonly used to enhance proliferation and maintain epithelial cell types. EGF, together with basic fibroblast growth factor (bFGF; also known as FGF2) is also used to derive and expand ectodermal lineages, including epidermal cells and neural stem cells from embryonic stem cells (ESCs) and induced pluripotent cells (iPSCs) (Conti et al., 2005). EGF is further included during hepatoblast maturation in directed hepatic differentiation of human iPSCs (Mallanna & Duncan, 2013) and supports the expansion of pancreatic progenitors and their progression toward functional β-cell differentiation from human pluripotent cells (Zhang et al., 2009; Nostro et al., 2015).

Beyond its role as a defined supplement in cell culture workflows, EGF has broad therapeutic relevance in skincare and therapeutic applications, including the prevention of skin aging, wound healing, and clinical research in dermatology and regenerative medicine (Miller-Kobisher et al., 2021). EGF has been shown to treat diabetic foot ulcers, administered into wound sites, or used topically (Tsang et al., 2003; Fernández-Montequín et al., 2007), and to promote the osteogenic differentiation of dental pulp stem cells through increased extracellular matrix mineralization, supporting its use in stem cell-based therapy for bone tissue engineering, including those involving mesenchymal stromal cells (MSCs) (Del Angel-Mosqueda et al., 2015). In the context of MSCs specifically, EGF enhances proliferation, multipotency preservation, and paracrine secretory activity, supporting MSC expansion without driving spontaneous differentiation and improving their therapeutic potential in regenerative applications (Krampera et al., 2005; Tamama et al., 2010). More recently, EGF has been used to enhance MSC-derived extracellular vesicle (EV) production, where EGF, along with TNF-alpha and hypoxia, significantly increases MSC-EV yield while preserving MSC stemness and improving EV-mediated wound healing (Chen et al., 2025).

The diverse biological effects of EGF are mediated through a well-characterized receptor signaling axis. Upon binding to epidermal growth factor receptor (EGFR1; also known as ErbB-1), EGF induces receptor autophosphorylation, which activates downstream signaling cascades, including the PI3K/Akt/mTOR and MAPK/ERK pathways, driving gene expression patterns associated with cell metabolism, growth, and division (Lavoie et al., 2020; Wee & Wang, 2017). Because ERK phosphorylation occurs rapidly and in proportion to EGFR activation, pERK is a widely used readout for quantifying the bioactivity of recombinant EGF preparations.

Here, we have evaluated the biological activity of OptiKine EGF by assessing its ability to induce phosphorylation of ERK, characterizing its dose-dependent potency in a cell proliferation assay, and measuring its ability to support Vero cell growth in a defined cell culture system.

RESULTS AND DISCUSSION

OptiKine EGF Activity is Demonstrated by Phosphorylation of ERK

To verify the biological function of OptiKine EGF, activation of the MAPK/ERK pathway was assessed by measuring total ERK and phosphorylated ERK (pERK) following acute EGF stimulation. BALB/3T3 cells, a mouse fibroblast line widely used to measure EGF potency (Kimball et al., 1988), were serum-starved to inhibit ERK signaling by other serum growth factors and treated with no EGF or OptiKine EGF at 10 or 20 ng/mL. Cell lysates were analyzed by capillary-based western blotting (Wes) using antibodies specific for pERK and total ERK (**Figure 1A**).

Treatment with OptiKine EGF at both 10 and 20 ng/mL produced a clear increase in pERK signal relative to untreated controls, while total ERK levels remained consistent (**Figure 1B**). Quantification of the pERK to total ERK ratio demonstrated a significant induction of ERK phosphorylation upon EGF treatment in a dose-dependent manner (**Figure 1C**). These results demonstrate that OptiKine EGF retains its activity by initiating intracellular signaling cascades associated with cell proliferation and survival.

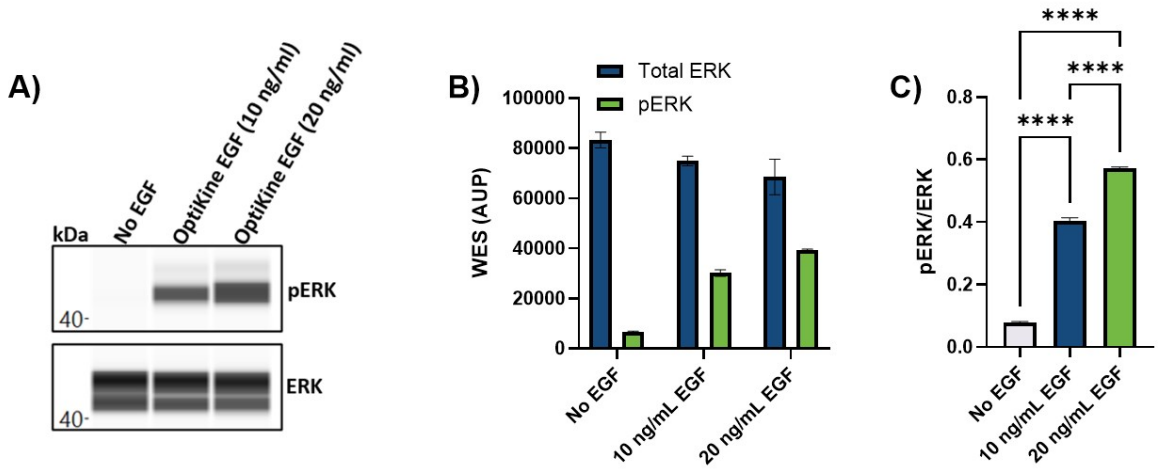
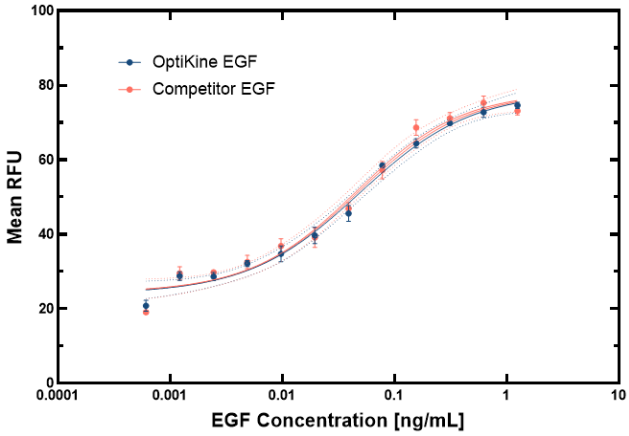


Figure 1. *Characterization of ERK phosphorylation by capillary western blot in response to OptiKine EGF. A) Capillary western blot image of total ERK and phosphorylated ERK (pERK) in serum-starved BALB/3T3 lysates in the absence or presence of 10 or 20 ng/mL OptiKine EGF. B) Quantitation of the band intensities from (A). C) Quantitation of the ratios of pERK/ERK band intensity in each lane from (A). Differences in pERK/ERK ratio between EGF statistical comparisons were assessed by ordinary one-way ANOVA with Tukey's multiple comparisons test. **** $p < 0.0001$, by one-way ANOVA with Tukey's post hoc test.*

OptiKine EGF Exhibits Comparable Bioactivity to Commercial EGF

OptiKine EGF exhibited an EC_{50} of 0.049 ng/mL with a 95% confidence interval of 0.037 to 0.068 ng/mL, while the competitor EGF produced an EC_{50} of 0.045 ng/mL with a 95% confidence interval of 0.033 to 0.062 ng/mL. Both dose-response curves were well fit by four-parameter logistic regression with R^2 values for each curve greater than 0.97, and the confidence intervals overlap for the EC_{50} values, indicating comparable potency observed in the activity of the two EGF sources. The observed EC_{50} values are consistent with the expected potency range for EGF (Rubin et al., 1991) and confirm that OptiKine EGF induces mitogenic activity comparably to a leading competitor standard.



	EC_{50} (ng/mL)	95% CI (ng/mL)	R^2
OptiKine EGF	0.0492	0.0373–0.0683	0.9782
Competitor EGF	0.0453	0.0332–0.0620	0.9726

Figure 2. *Bioactivity determination of OptiKine EGF and competitor EGF in a BALB/3T3 cell proliferation assay. Proliferative response was measured across a titration of OptiKine EGF or competitor EGF and fit by four-parameter logistic regression. Data points represent mean ± SD; n = 3 technical replicates per concentration. EC_{50} values, 95% confidence intervals (CI), and R^2 values are reported in the inset table.*

Vero cells, a cell line derived from the kidney of the African green monkey, Chlorocebus aethiops, are widely used in virology and vaccine development (Govorkova et al., 1996). Because vaccine manufacturing increasingly relies on serum-free, chemically defined systems to reduce variability and biosafety risk, EGF can provide critical mitogenic support under these conditions. To evaluate the ability of OptiKine EGF to support cell expansion in a fully defined system, Vero cells were cultured in InVitria's serum-free OptiVERO media supplemented with OptiKine EGF, competitor EGF, or no EGF, and fold expansion was monitored over three days (**Figure 3A**). OptiKine EGF supplementation drove robust Vero proliferation, reaching 11.5-fold expansion by day 3, comparable to the competitor EGF (10.2-fold) and significantly greater than the no-EGF control (3.9-fold) (**Figure 3B**). These data establish that OptiKine EGF supports robust Vero cell expansion in a fully defined system suitable for serum-free vaccine manufacturing workflows.

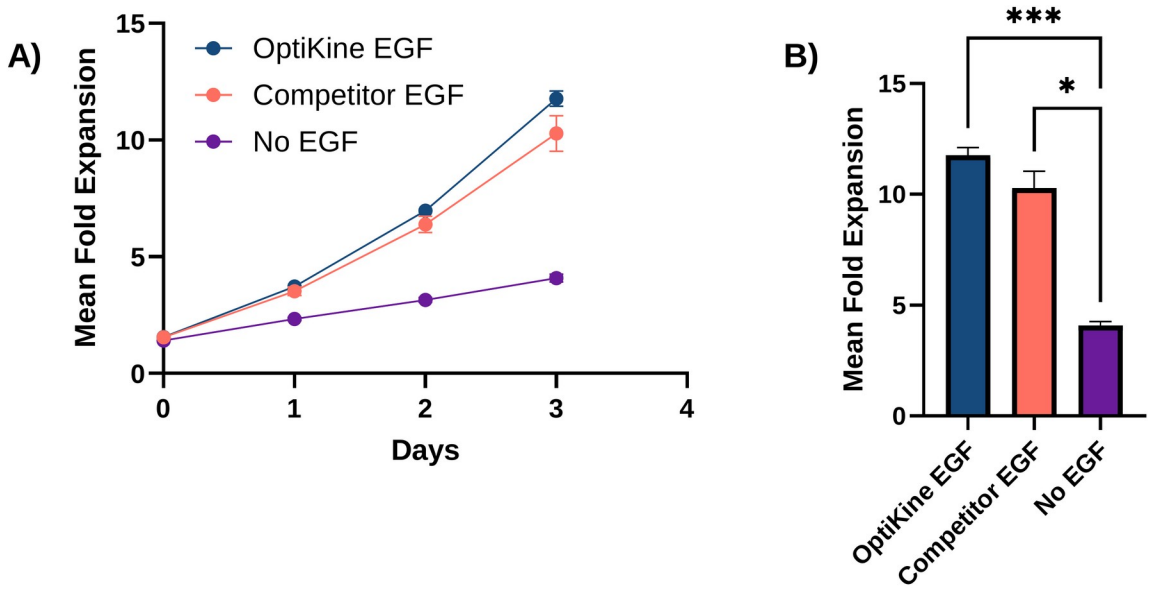


Figure 3. *Assessment of the effect of EGF on Vero cell expansion. A) Mean fold expansion of Vero cells in OptiVERO serum-free media over three days in the absence or presence of OptiKine EGF or competitor EGF. B) Cumulative fold expansion on day 3. Group differences were evaluated by Brown-Forsythe and Welch ANOVA. ***p < 0.001, *p < 0.05.*

CONCLUSION

InVitria's OptiKine EGF was shown to have biological activity by inducing ERK phosphorylation under serum-starved conditions. Quantitative potency testing demonstrated mitogenic activity equivalent to a leading competitor EGF, with comparable EC_{50} values and overlapping confidence intervals in a BALB/3T3 proliferation assay. In a defined serum-free cell culture system, OptiKine EGF supported robust, EGF-dependent Vero expansion comparable to the competitor product and far exceeding the no-EGF control, confirming like-kind performance for serum-free vaccine manufacturing and highlighting the importance of EGF in the culture of responsive cell lines. In a sterile, ready-to-use liquid format manufactured in an animal-free platform, OptiKine EGF combines high purity and GMP-grade quality with reduced endotoxin and host cell protein risk. Collectively, these data demonstrate that OptiKine EGF is a reliable, animal-origin-free recombinant EGF that performs comparably to existing products while enabling defined, scalable Vero cell culture.

MATERIALS AND METHODS

Cell Culture

BALB/3T3 clone A31 cells (ATCC CCL-163) and Vero cells (ATCC CCL-81) were maintained at 37 °C in a humidified incubator with 5% CO_2. BALB/3T3 cells were cultured in DMEM (Gibco 11964) supplemented with 10% bovine calf serum; Vero cells were cultured in DMEM/F12 (Gibco 11320) supplemented with 10% fetal bovine serum.

ERK Phosphorylation by Simple Western (Wes)

To assess EGFR pathway activation, BALB/3T3 cells were serum-starved in DMEM/F-12 supplemented with 1× ITS (InVitria 777ITS091) and 10 mM HEPES, then stimulated with EGF for 15 min. BALB/3T3 cells were then lysed in lysis buffer containing phosphatase and protease inhibitors, and lysates were analyzed on a capillary-based Simple Western system (Wes, ProteinSimple) using antibodies specific for phosphorylated ERK1/2 (pERK) (Cell Signaling 9102S) and total ERK1/2 (Cell Signaling 9101S).

EGF Bioactivity by BALB/3T3 Proliferation Assay

EGF bioactivity was determined using a BALB/3T3 proliferation assay following the approach described by Rubin et al. (1991), measured by thymidine analog incorporation. Dose-response curves were fitted by four-parameter logistic regression to determine EC_{50} values.

Vero Cell Expansion in OptiVERO

Vero cells were seeded in a 6-well plate in OptiVERO base media (InVitria, 555OCM0039) supplemented with OptiKine EGF, a competitor EGF standard, or no EGF, and adapted to each condition for 3 days. Cells from each condition were then reseeded into 24-well plates at equal density (n = 3 per condition) in the same media and supplementation. Phase area confluence was monitored by live-cell imaging (Sartorius Incucyte SX5) over 3 days and normalized to the initial timepoint. Mean fold expansion was determined at days 1, 2, and 3.

Statistical Analysis

Group differences in mean fold expansion were evaluated by Brown-Forsythe and Welch ANOVA. Differences in pERK/ERK ratio between EGF statistical comparisons were assessed by ordinary one-way ANOVA with Tukey's multiple comparisons test. Data are mean ± SD from 3 independent biological replicates. A p-value < 0.05 was considered statistically significant. Specific tests are noted in figure legends.

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ABOUT INVITRIA

InVitria is a global leader in developing and manufacturing high-performance, animal-origin-free cell culture supplements and recombinant proteins. Our products eliminate human and animal serum-derived components to improve consistency, reduce regulatory burden, and accelerate the approval of life-changing therapies. Trusted in clinical and commercial production of cell and gene therapies, vaccines, regenerative medicine, and medical devices, InVitria supports cGMP and ISO-compliant manufacturing aligned with FDA and EMA expectations. Scalable to meet growing global demand, our chemically defined solutions enable safer, more efficient biomanufacturing.

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Performance. Defined.

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