

IMMOBAZYME - INSTRUCTION MANUAL



EPIDERMAL GROWTH FACTR (HUMAN)

PRODUCT OUTLINE

PRODUCT NAME

Epidermal Growth Factor (Human)

MANUFACTURER

Immobazyme (Pty) Ltd

BATCH DETAILS

CAS: 106096-93-9 | LOT: 20250123-EGF

Product Specification & Protocol Epidermal Growth Factor (Human)

PRODUCT INFORMATION

Epidermal Growth Factor, also known as EGF, is a potent growth factor primarily synthesised by epithelial cells and platelets. As a member of the EGF family, it binds specifically to the Epidermal Growth Factor Receptor (EGFR) to activate intracellular signalling pathways. These protein-protein interactions play a crucial role in regulating cellular proliferation, differentiation, and migration.

Immobazyme's recombinant EGF is produced in *E. coli*, and purified using immobilised metal ion affinity chromatography. Immobazyme's EGF is a fusion protein with enhanced stability and solubility.

PRODUCT SPECIFICATION

For Research Use only

Grade	Food-grade, Allergen-free
Amount	100 µg per vial
Molecular Weight	23.9 kDa (with fusion protein)
Production System	<i>E. coli</i>
Protein Information	Recombinant EGF is a monomeric fusion peptide with enhanced stability and solubility
Purification Method	Sequential chromatography (IMAC and desalting)
Filtration	Filtered through a 0.22 µm sterile filter
Sterility	Sterile
Mycoplasma	Absent
Form	Lyophilised powder
Purity	>95%
Formulation	10 mM Na ² HPO ₄ , 1.8 mM KH ² PO ₄ , 2.7 mM KCl, 100 mM NaCl, pH 7.0, 2% Dextran T40

RECONSTITUTION PROTOCOL AND STORAGE

Reconstitute EGF

Perform reconstitution in a sterile laminar flow hood.

1. Remove red safety cap from vial.
2. Aspirate 1 mL of sterile milliQ water into a 1 mL sterile syringe.
3. Attach a sterile needle onto the syringe and insert into the vial through the centre of the rubber stopper seal.
4. Gently inject the 1 mL of water into the vial, then remove the needle and syringe.
5. Invert the vial 5-10 times, or until the lyophilised sample is fully reconstituted.
6. Insert the needle and syringe into the reconstituted sample vial, invert the vial and gently aspirate the sample liquid into the 1 mL syringe, being sure to collect the full volume by keeping the needle end near the rubber stopper opening.
7. Inject the reconstituted 1 mL sample into a sterile microfuge tube through a 0.22 µm syringe filter (provided).
8. Prepare stock concentrations in sterile microfuge tubes as per your relevant standard operating procedures, keeping in mind the avoidance of repeated freeze-thaw cycles.
9. Prepare working concentration stocks in sterile microfuge tubes as per your relevant standard operating procedures. The recommended working concentration for hEGF is 1-100 ng/mL.

Storage Instructions:

- The lyophilised vial can be stored at -20 °C for 12 months.
- The reconstituted protein aliquots can be stored at -20°C for 6 months.
- Once resuspended use within 1 week (storage at 4°C).

Important Notes:

- Avoid repeated freeze-thaw cycles.

QUALITY CONTROL & PERFORMANCE TESTING

Purity verification: SDS-PAGE and Coomassie staining

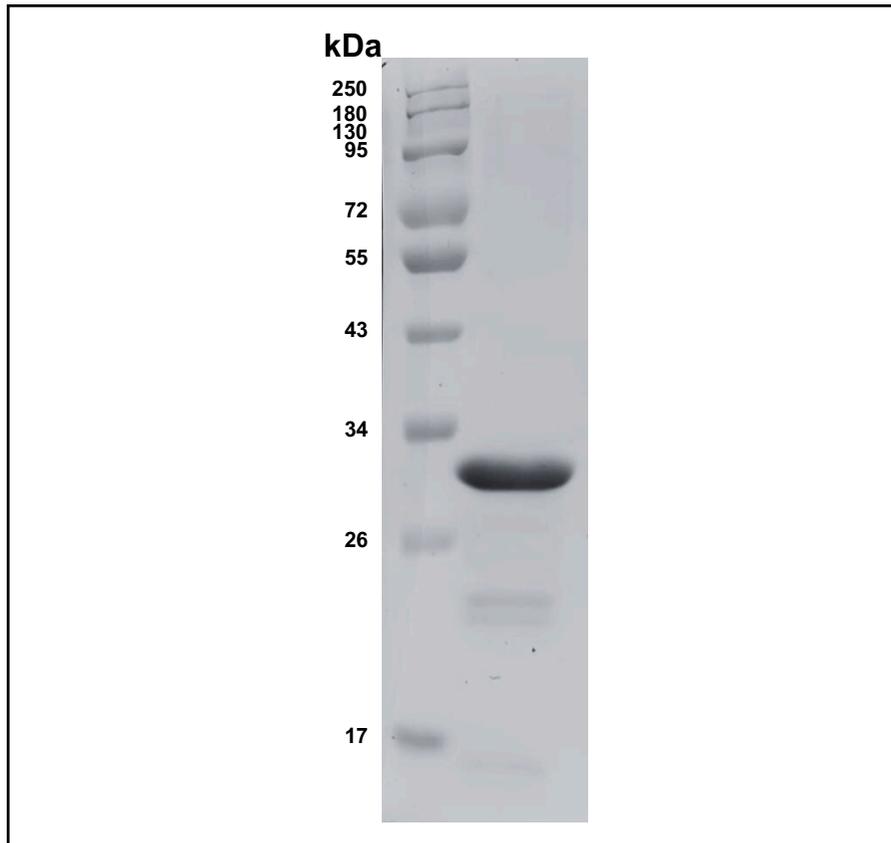


Figure 1. EGF (LOT: 20250123-EGF) run on an SDS-PAGE gel after lyophilisation. A prominent band representing EGF was present with $\pm 90\%$ purity.

Effect of EGF on HaCaT cell proliferation

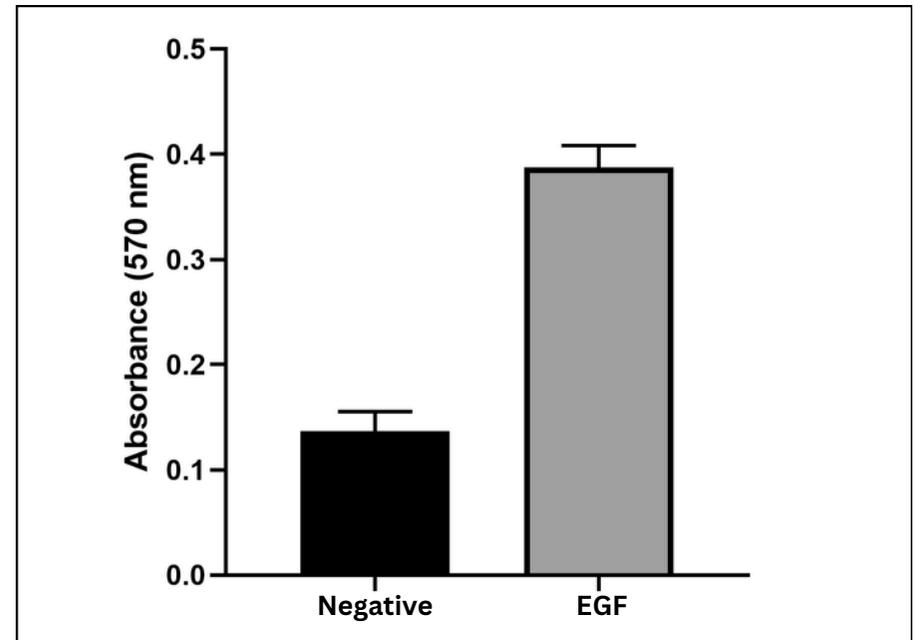


Figure 2. Effect of EGF (20250123-EGF) on HaCaT keratinocyte cell proliferation, tested over 48 hours.

This bar graph illustrates the effect of EGF on the proliferation of HaCaT keratinocyte cells after 48 hours. The y-axis represents the absorbance value measured at 570 nm, indicating relative cell proliferation. The x-axis presents the tested samples; a negative control of HaCaT cells in the absence of EGF, and HaCaT cells treated with 2.5 ng/mL of EGF. The results demonstrate a significant improvement in HaCaT cell proliferation when in the presence of EGF.

Absence of mycoplasma

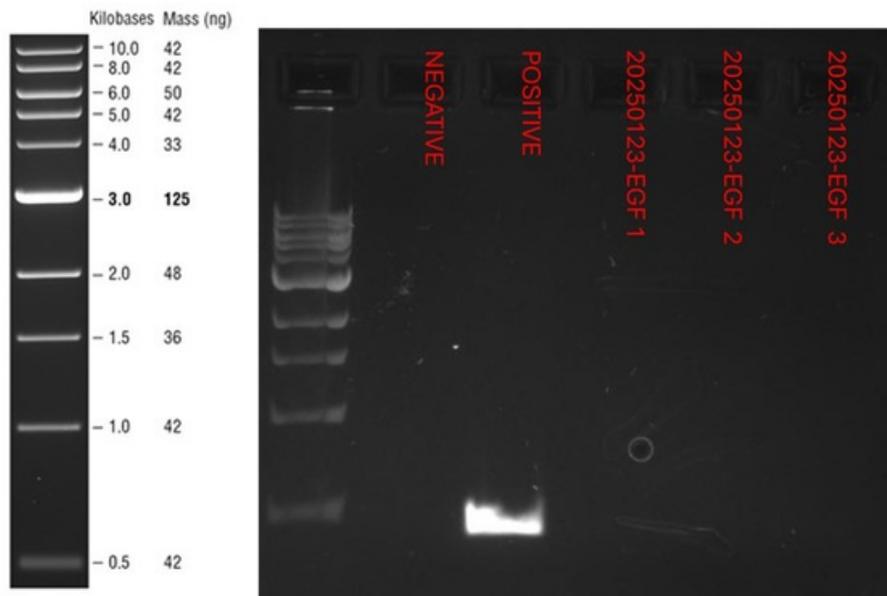


Figure 3. Mycoplasma detection via PCR on EGF (LOT: 20250123-EGF).

No amplification of the PCR product indicates that the samples are free of the mycoplasma contamination.

Observation of EGF treated HaCaT cells

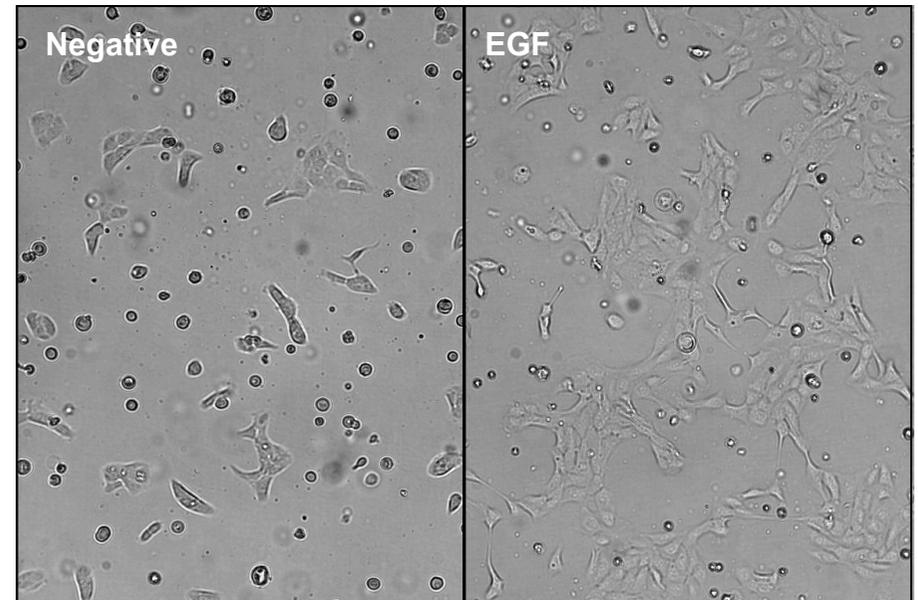


Figure 4. Microscopic observation of HaCaT cells treated with 2.5 ng/mL of EGF after 48 hours.

Not for diagnostic or therapeutic use.

Effect of EGF on cell migration

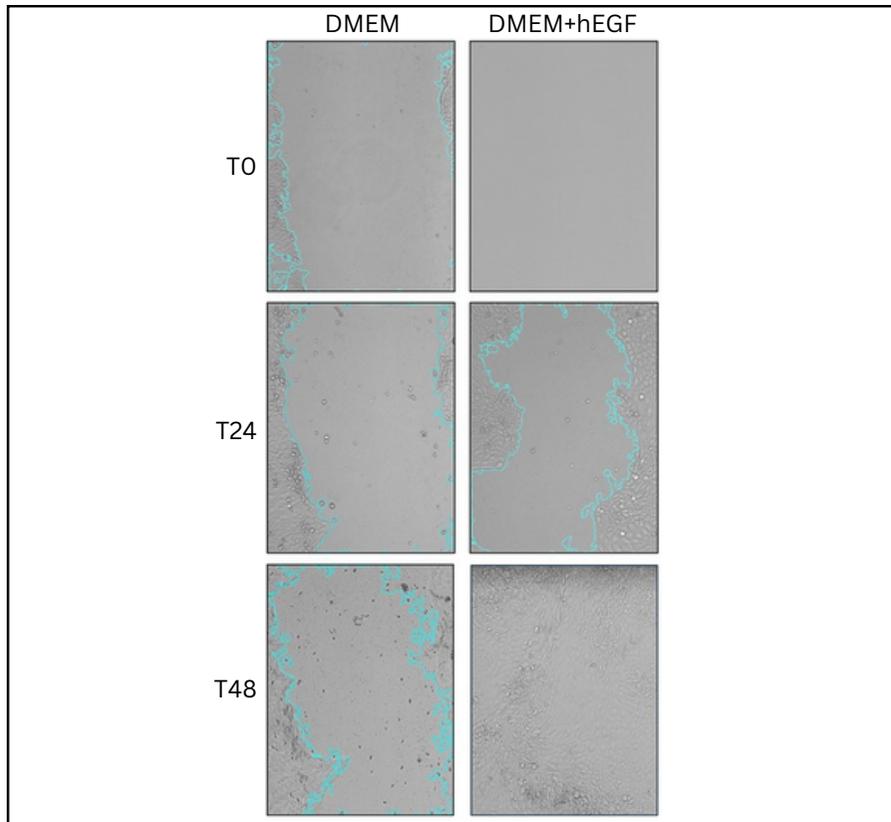


Figure 5. A scratch assay showing the effect of EGF on cell migration over 48 hours. Representative phase-contrast images were taken at 0 hours (T0), 24 hours (T24), and 48 hours (T48). The left column represents the control condition (DMEM), while the right column represents cells treated with DMEM supplemented with EGF. The initial wound area is outlined in blue. In the control condition, the wound closure is limited over time. In contrast, cells treated with hEGF exhibit significantly enhanced migration, resulting in near-complete wound closure by 48 hours. This demonstrates the pro-migratory effect of hEGF in promoting cell proliferation and wound healing.