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Product datasheet

Qk003 hFGF10: recombinant human FGF10

Summary

Mature domain of human FGF10 (residues 64-208, Uniprot: O15520) expressed in E.coli and purified to homogeneity.

Mature protein is a non-glycosylated protein with a molecular weight of 17 kDa.

Alternative names: Fibroblast Growth Factor-10, FGFA, KGF-2, Keratinocyte growth factor 2

Form

Protein is provided in PBS without carrier protein. The concentration is 1 mg/ml.

Sequence

M G R H V R S Y N H L Q G D V R W R K L F S F T K Y F L K I E K N G K V S G T K K E N C P Y S I L E I T S
V E I G V V A V K A I N S N Y Y L A M N K K G K L Y G S K E F N N D C K L K E R I E E N G Y N T Y A S F N
W Q H N G R Q M Y V A L N G K G A P R R G Q K T R R K N T S A H F L P M V V H S*

Molecular weight

Expected MW: 16912.4 Da

Handling guidance

Thaw the sample on ice, spin briefly and dilute with PBS as needed. Spin in a microfuge for 5 minutes at maximum speed, and divide the solution into suitable aliquots and store at -80°C. We recommend that single-use aliquots should be prepared to avoid freeze-thaw cycles. Every effort is made to ensure samples are not contaminated in any way however we recommend sterile filtering after dilution in media or the final working solution.

By defining very strict and relevant quality control criteria, we provide proteins that work exactly the same way, every day, from batch to batch, at any scale you need. Batches that do not match these strict criteria are not accepted for sale.

Simple as that.

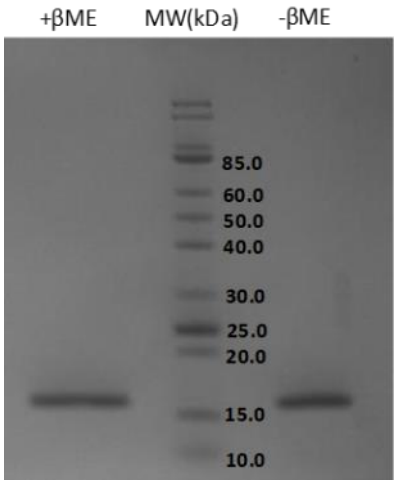
Batch specific quality testing

Qk003 hFGF10 batch #010

We take the quality of our cytokines and growth factors very seriously. All our proteins are produced in-house by our scientists and we understand the potential impact on your work if your cytokines are not consistent and of the highest quality. If you have any questions about our proteins or QC data, please email support@qkine.com or you can contact Catherine directly at catherine@qkine.com.

Purity: SDS-PAGE

The protein migrates as a single band at ca. 17 kDa in non-reducing (-βME) conditions and upon reduction (+βME).

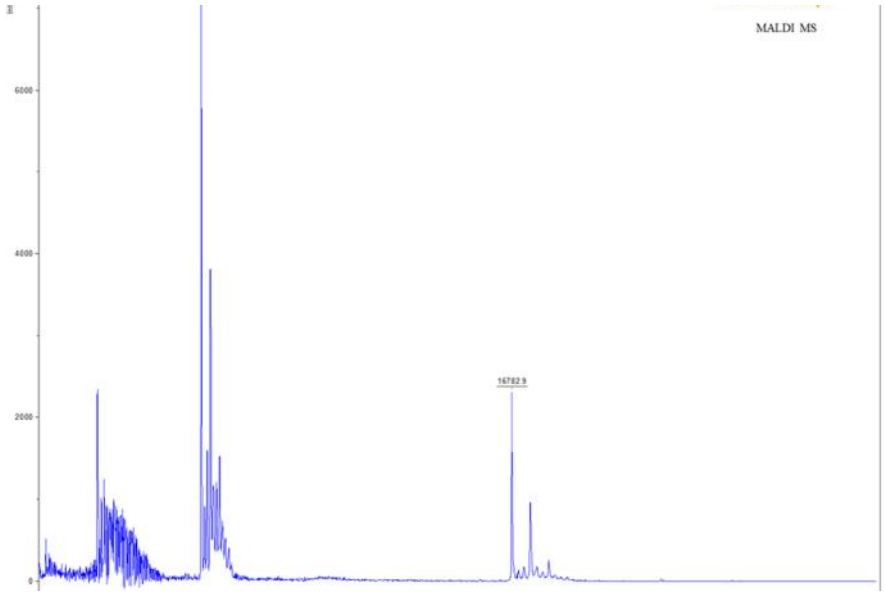


FGF10 #010 purity confirmed using SDS-PAGE
FGF4 #010 protein (7 µg) was resolved using 15% w/v SDS-PAGE in reduced (+β-mercaptothanol, +βME) and non-reduced conditions (-βME) and stained with Coomassie Brilliant Blue R250.

Purity and identity: mass spectrometry

MALDI mass spectrometric analysis to confirm expected molecular mass of the intact protein, with the assumption that all the cysteines are disulphide linked. There are no minor contaminants.

Expected MW: 16912.40 Da
Result analysis: 16781.21 Da
Result confirms molecular weight of FGF10



Mass spectrometry analysis of hFGF10 #010 shows protein at expected molecular mass.

hFGF10 #010 in 100 mM sodium phosphate pH 7.4 was analysed by an external service provider. The different peaks represent different charge states of the protein. These are used to calculate the mass of the protein, which is then compared to the calculated theoretical mass.

Batch specific quality testing

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Endotoxin analysis

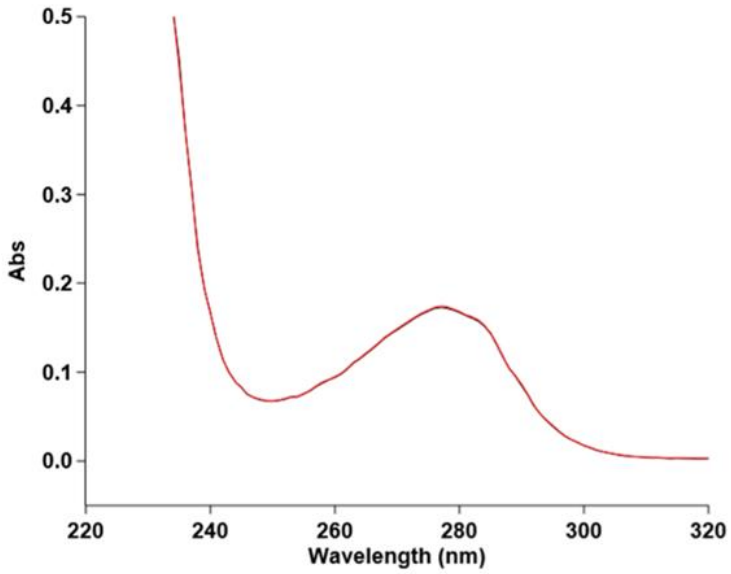
Stem cell cultures are sensitive to endotoxins[1], which can be present in media, serum and as a contaminant on plasticware. We optimise our protein production processes to ensure the lowest possible levels of endotoxin contamination. Our endotoxin pass criteria are set at the industry leading <0.1 EU per ug protein and we aim for <0.01 EU per ug protein. Endotoxin levels in our proteins are determined by an external expert microbiological testing services provider.

hFGF10 batch #010 endotoxin level <0.005 EU/ug protein (below level of detection)

Recovery

After aliquoting, we test a unit of stock to ensure full protein recovery

Absorbance at 280 nm: average 0.172
Recovered concentration: 0.172 cm^{-1} x 10 / 1.44 cm^{-1} mg ml^{-1} = 1.19mg / ml
Recovery: >100% (due to routine over-fill of vials during aliquoting)



UV spectrum of FGF10 #010 shows full recovery of protein following aliquoting

The sample was diluted 1:10 in 100 mM sodium phosphate pH 7.4 and the UV spectrum 340-220 nm measured in duplicate (orange and black line). Concentration was calculated using extinction coefficient at 280 nm

References

1. A biological study establishing the endotoxin limit for in vitro proliferation of human mesenchymal stem cells (2017). Yusuke Nomura, Chie Fukui, Yuki Morishita, Yuji Haishima. Regenerative Therapy, 7, 45-51.

Additional data

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Bioactivity

Epithelial to mesenchymal transition (EMT) is a crucial morphogenetic process during development in which cells lose their epithelial characteristics and acquire migratory mesenchymal properties. FGF10 has an important role both during the embryonic EMT (type I) and on cancer cell initiation of metastasis (type III EMT).

Keratinocyte culture

Qk003 hFGF10 (batch #010) supports proliferation and promotes epithelial to mesenchymal transition in human primary keratinocyte proliferation. Data and evaluation by Stemnovate Ltd.

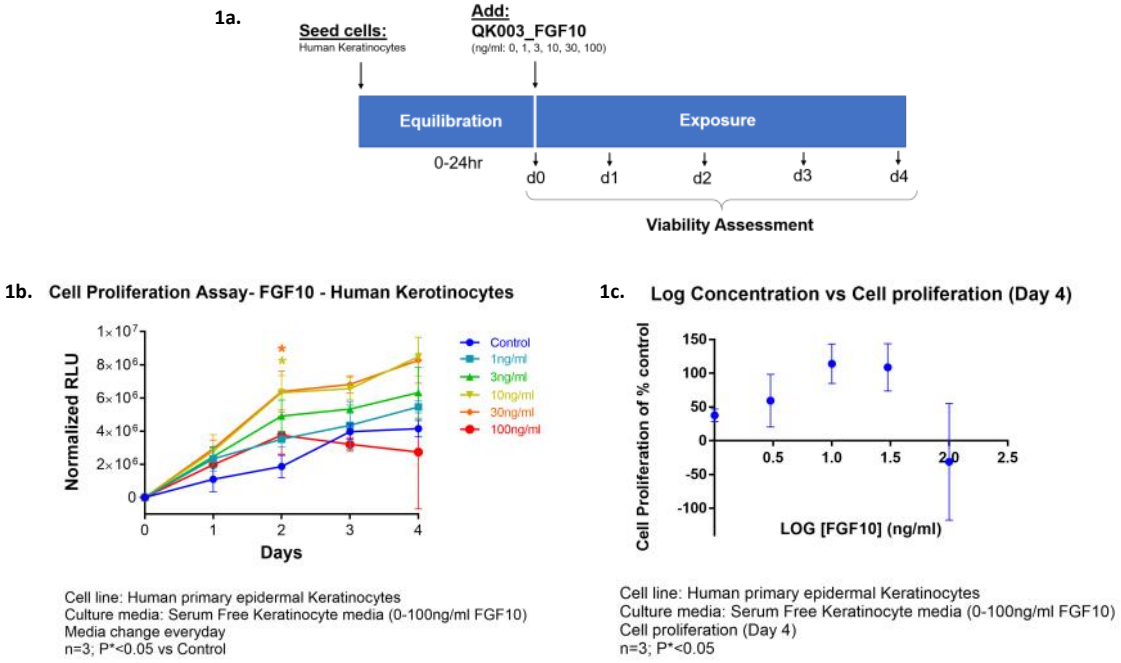

Figure 1. Cell proliferation assays to assess the effect of Qkine FGF10 (0-100 ng/ml) on human primary epidermal keratinocytes in serum-free keratinocyte media. Cells were evaluated at culture days: 0 (baseline), 1, 2, 3, 4 days, as summarized schematically in Figure 1a. Figure 1b shows cell proliferation (Relative Luminescence Unit [RFU]) for days 1, 2, 3, 4 and normalized to day 0 readouts (n=3; P*<0.05 vs control). The log concentration plot in Figure 1c shows percent cell proliferation normalized over untreated control (%) and to day 0 (baseline) after 4 days treatment (n=3; P*<0.05). The maximal cell proliferation was observed at ~10ng/ml FGF10 and a reduction in cell number/viability as observed at 100 ng/ml. Data provided by Stemnovate Ltd, Cambridge, UK.

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Keratinocyte culture

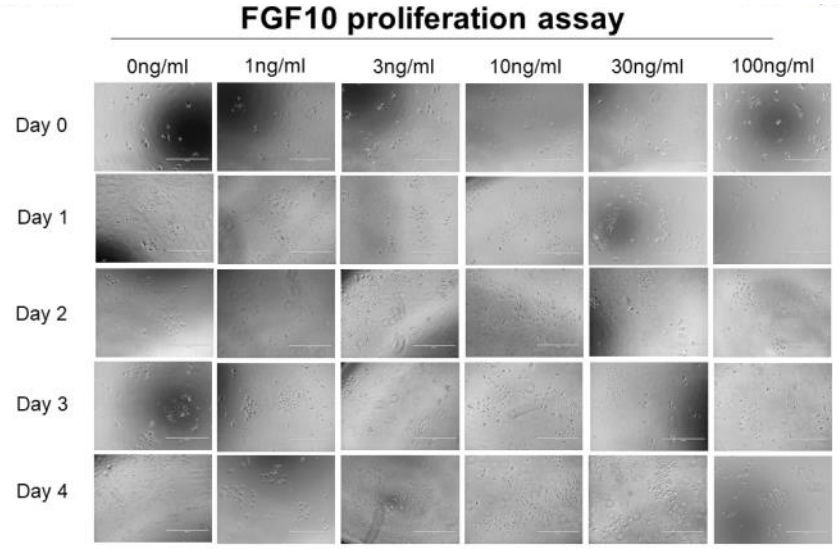

Figure 2. Representative images showing human primary epidermal keratinocytes treated with Qkine FGF10 at (0-100 ng/ml) at d0 (baseline), d1, d2, d3 and d4.

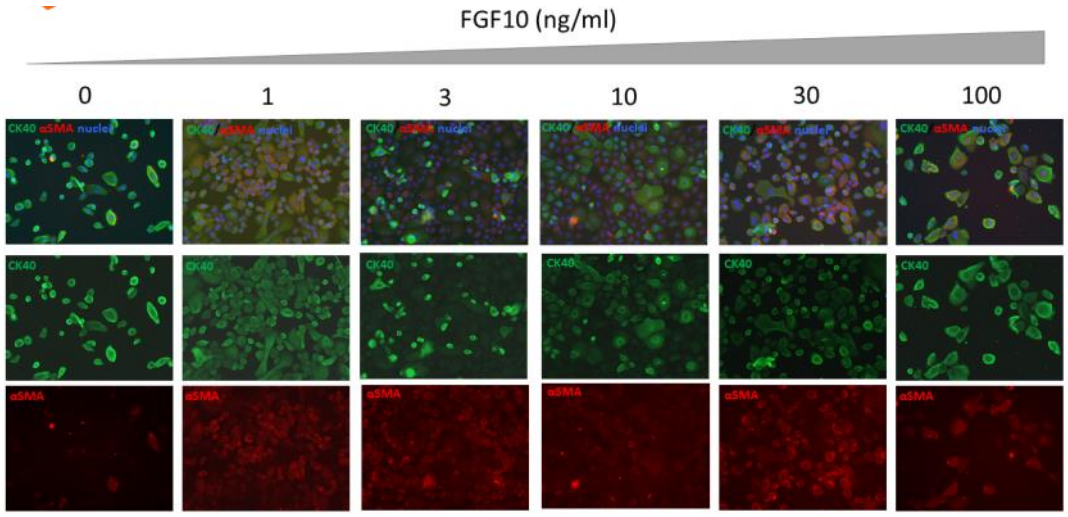

Figure 3. Induction of EMT in human primary keratinocytes following treatment with hFGF10. Induction of EMT was evaluated using immunofluorescence staining to determine expression of the epithelial marker (Cytokeratin 14 [CK14]) and mensenchymal marker (α-Smooth Muscle Actin [αSMA]) in Human primary epidermal keratinocytes after 4 days treatment with Qk003 hFGF10 (0-100 ng/ml).