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QK002_FGF2: basic FGF (FGF2)

Description

Mature domain of zebrafish *Danio rerio* FGF2 (residues 2-154, Uniprot: B3DGE3) expressed in *E.coli* and purified to homogeneity as described in Ludwig *et al*. (2006)[1,2].

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

Protein is provided in PBS without carrier protein at 10mg/ml.

Sequence:

ATGGITTLPPAPDAENSSFPAGSFRDPKRLYCKNGGFFLRINADGRVDGARDKNDPHIRL

QLQATAVGEVLIKGICTNRFLAMNADGRLFGTKRTTDECYFLERLESNNYNTYRSRKYPD

WYVALKRTGQYKSGSKTSPGQKAILFLPMSAKC*

Handling instructions:

Thaw the sample on ice 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. They can remain at -80°C for up to 10 months. At -20°C, the protein can be stored for up to 3 months without reduction in the activity. We recommend that single-use aliquots should prepared to avoid freeze-thaw cycles.

The sample is not sterile, and whilst every effort is made to ensure they are not contaminated in any way, we would recommend sterile filtering after dilution in media or the final working solution.

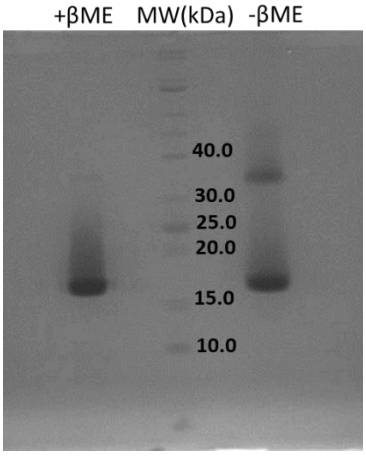
Quality control

Batch: QK002_FGF2.010

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 Catherine (catherine@qkine.com).

Purity: SDS-PAGE

The protein elutes as major band at ca. 17 kDa in non-reducing (-βME) conditions and upon reduction (+βME). The higher molecular mass band at ca. 35kDa is a dimer that we always see in our highly purified FGF2, the presence of this does not affect biological activity.



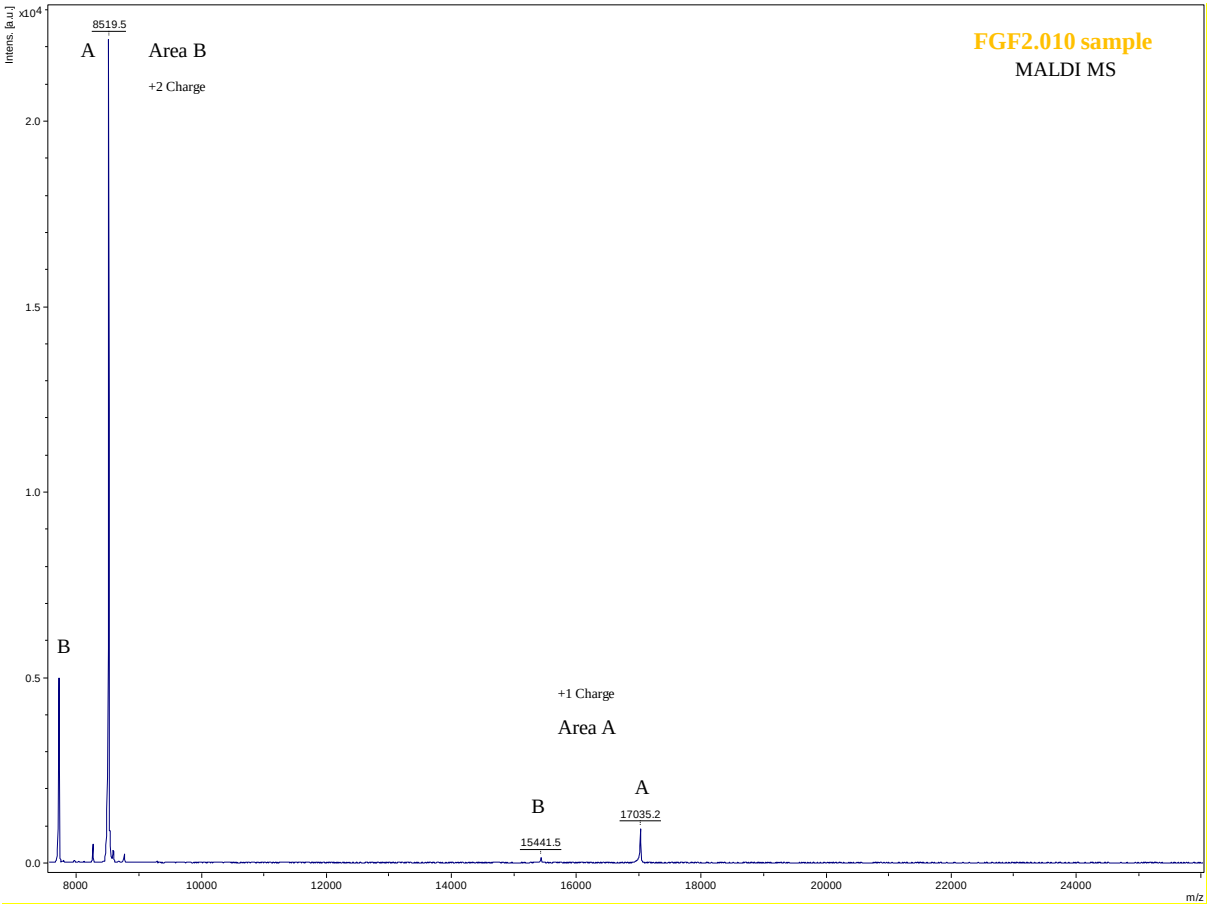
Analysis of the purity of FGF2.010 using SDS-PAGE

FGF2.010 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 assumption that all the cysteines are disulphide linked and there are no minor contaminants.

Expected MW: 17245 Da
Result analysis: 17035 Da
Result confirms molecular weight of FGF2



Mass spectrometry analysis of FGF2.010 shows protein at expected molecular mass.

FGF2.010 in 10 mM HCl 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.

Purity – endotoxin testing

Endotoxin levels are determined by an external expert microbiological testing services provider

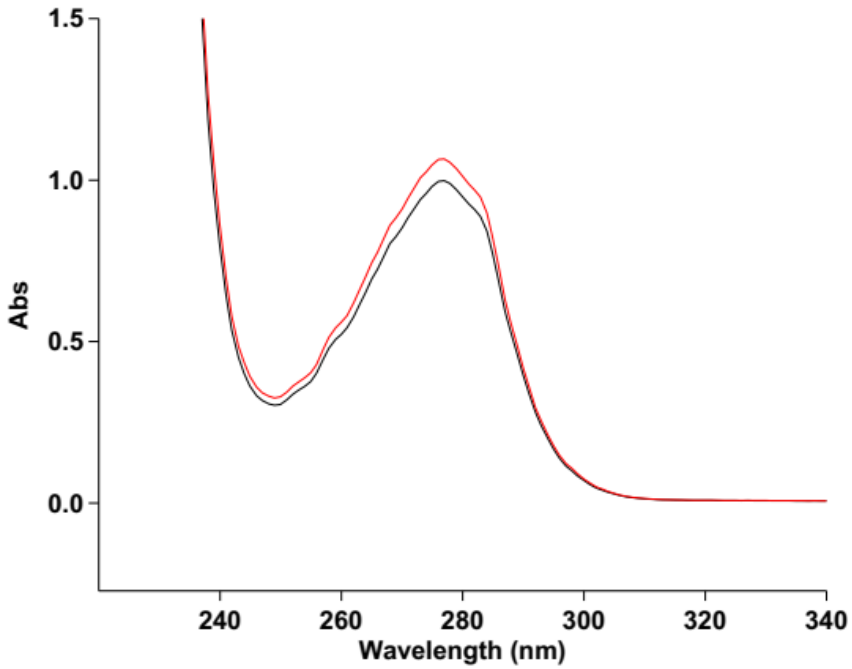
Stem cell cultures are sensitive to endotoxins[3], which can be present in media, serum and as a contaminant on plasticware. We optimise our protein production processes to ensure the lowest possible levels of endotoxin contamination. We set our endotoxin pass criteria at the industry leading <0.1 EU per ug protein and aim for <0.01 EU per ug protein.

FGF2 batch 010 endotoxin level = <0.005 EU/ug protein

Recovery

A unit of stock is tested to ensure full protein recovery

Absorbance at 280 nm: average 0.980
Recovered concentration: 0.980 cm^{-1} x 10 / 0.935 cm^{-1} mg ml^{-1} = 10.48 mg / ml
Recovery: >100% (due to routine over-fill of vials during aliquoting)



UV spectrum of FGF2.010 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. Ludwig, T. E. *et al.* Feeder-independent culture of human embryonic stem cells. *Nat. Methods* **3,** 637–646 (2006).
2. Ludwig, T. E. *et al.* Derivation of human embryonic stem cells in defined conditions. *Nat. Biotechnol.* **24,** 185–187 (2006).
3. Nomura, Y., Fukui, C., Morishita, Y. & Haishima, Y. A biological study establishing the endotoxin limit for in vitro proliferation of human mesenchymal stem cells. *Regen. Ther.* **7,** 45–51 (2017).

The team at Qkine are always happy to help. Please contact us orders@qkine.com or +44 (0) 1223 491486 if you have any questions.

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