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Product datasheet

Qk017 hGDF15: recombinant human GDF15

Summary

Mature domain of human GDF15 (Uniprot: Q99988) expressed in *E.coli* and purified to homogeneity.

Mature active protein is a disulphide-linked dimer in with a molecular weight of 25 kDa.

Alternative names: Macrophage inhibitory cytokine 1 (MIC 1), NSAID activated gene 1 protein (NAG1),

Form

Protein is provided in lyophilised form without carrier protein.

Sequence

MARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANMHAQIKTSLHRLKP
DTVPAPCCVPASYNPMVLIQKTDTGVSLQTYDDLLAKDCHCI

Molecular weight

The active dimer should be 24556.4 Da by mass-spec, with N-terminal methionine cleaved, and all disulphides (9 per dimer) formed.

Handling guidance

GDF15 is poorly soluble and sticky. Physiological buffers will cause *massive* precipitation of stock solutions, hence we suggest storing them at very low pH to ensure solubility before dilution into cell culture media. Low pH will also help maintain the correct disulphide structure of the protein by minimising disulphide bond exchange reactions.

Dissolve GDF15 in 10 mM HCl (1:1000 dilution of concentrated HCl) while keeping the protein concentration at 50 µg/ml or above, in order to avoid loss by adsorption to plasticware. To ensure you recover all of the protein, let the sample sit for a few minutes with the solubilisation buffer at room temperature and pipette gently up and down (avoid foaming). Rinse the tube with some more 10 mM HCl and pool with the rest. The protein is tolerant of some freeze and thaw cycles, but as always with proteins, it is better to aliquot and stored frozen. If possible, add carrier protein of your choice such as BSA, HSA or gelatin to further minimise loss by adsorption. Please be cautious about contamination from other growth factors if using a carrier protein. Store in -80°C for long term storage. -20°C is fine for short-term.

Every effort is made to ensure samples are sterile 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.

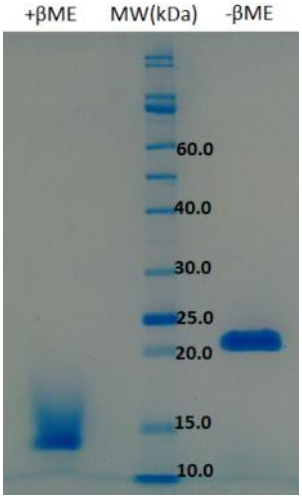
Batch specific quality testing

Qk017 hGDF15 batch #011

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, our CEO, directly at catherine@qkine.com.

Purity: SDS-PAGE

The protein migrates as a single band at ca. 24 kDa in non-reducing (-βME) conditions and 13 kDa upon reduction (+βME).

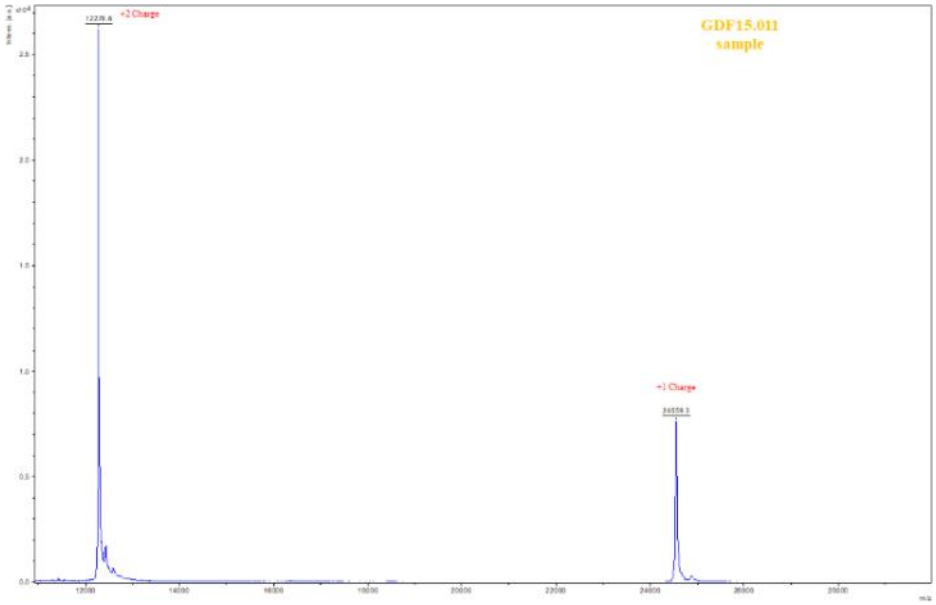


GDF #011 purity confirmed using SDS-PAGE
GDF15 #011 protein (7 µg) was resolved using 15% w/v SDS-PAGE in reduced (+β-mercaptothanol, +βME) and non-reduced conditions (-βME) and stained with Coomassie Brilliant Blue R250.

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: 24556.4 Da
Result Analysis: 24556.8 Da
Result confirms molecular weight of GDF15



Mass spectrometry analysis of hGDF15 #011 shows protein at expected molecular mass.

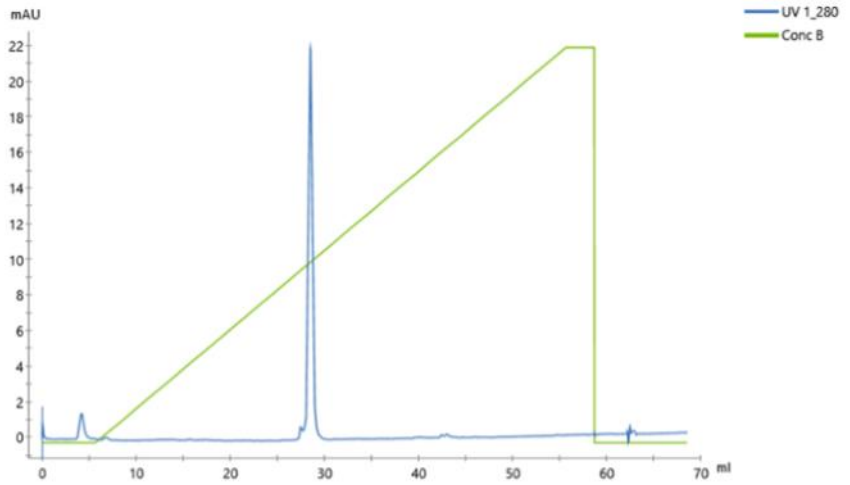
hGDF15 #011 in 100 mM sodium phosphate pH 7.4 was analysed by an external service provider. The different peaks represent different charge states of the protein. These are used to calculate the mass of the protein, which is then compared to the calculated theoretical mass.

Batch specific quality testing

Qk017 GDF15 batch #011

Purity: analytical reverse phase

Analysis of protein purity and homogeneity, judged by the absence of multiple peaks and the profile of the GDF15 peak.



Reverse phase chromatogram of GDF15 #011 shows single sharp peak.

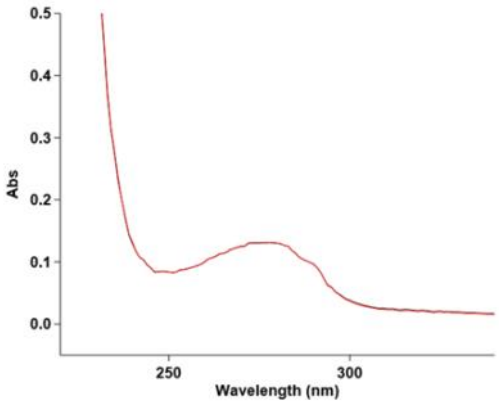
50 µg of GDF15 batch #01 was diluted in 10 mM HCl to 0.1 mg/ml and run in an analytical ACE C4 4.6 x 250 mm column at 1 ml/min and eluted using a 10 – 90 % acetonitrile gradient in 0.1 % trifluoro acetic in 65 minutes.

Blue line shows absorbance at 280 nm and the green line the acetonitrile gradient. Protein eluted in a sharp single peak which confirms that QK017 GDF15 has a very high level of purity and homogeneity.

Recovery

A unit of stock is tested to ensure full protein recovery following reconstitution

Absorbance at 280 nm: average 0.13
Recovered concentration: 0.13cm-1 x 10 / 1.17 cm-1 mg ml-1 =1.1mg / ml
Recovery: 110% (>100% due to routine 10% over-fill of vials during aliquoting)



UV spectrum of GDF15 batch #011 shows full recovery of protein following aliquoting and lyophilisation

The sample was reconstituted in 10 mM HCl to a theoretical concentration of 1 mg/ml following instructions above. This was diluted 1:10 in 6 M guanidine hydrochloride, 20 mM sodium phosphate pH 7.4 and the UV spectrum 340-220 nm. Concentration was calculated using extinction coefficient at 280 nm.

Batch specific quality testing

Qk017 GDF15: recombinant human GDF15

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.

hGDF15 batch #011 endotoxin level <0.005 EU/ug protein (below level of detection)

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

Qk017 hGDF15: recombinant human GDF15

Bioactivity

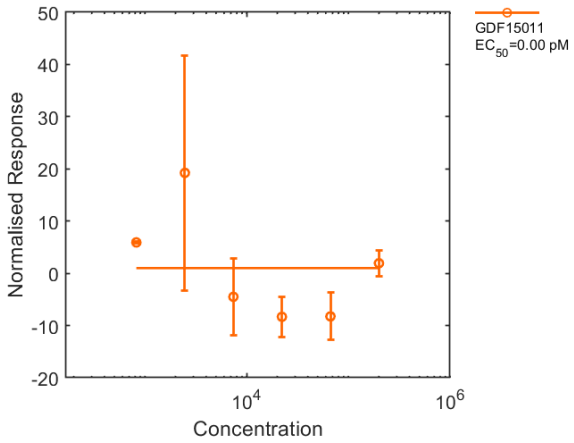
GDF15 signals through GRAL and co-receptor RET leading to RET phosphorylation and signalling through the ERK and AKT pathway (reviewed in Emmerson *et al.*, 2018). Commercial sources of GDF15, in particular those purified from mammalian expression systems have been shown previously to be contaminated with trace amount of TGFβ. These trace contaminants cause misleading experimental results due to the picomolar or even femtomolar EC_{50}s (Olsen *et al.*, 2017). **Here we use a well characterised SMAD2/3 activation assay no contamination from TGFβ family proteins.**

No SMAD2/3 activation

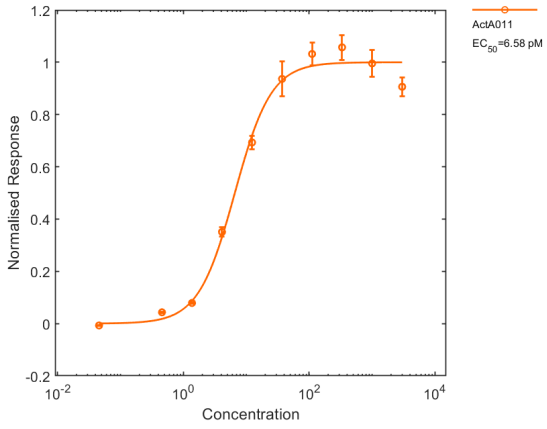
Purity of Qk017 GDF15 preparations #011 was determined using an SMAD2 /3 responsive luciferase reporter in HEK293T cells and compared to a positive control (Activin A Qk001)

Result: EC_{50} = 0 pM (no contamination with TGFβ or related growth factors)

1a.



1b.



Qk017 GDF batch #011 shows no TGFβ contamination.

HEK293T cells were transfected with TGFβ / Activin-responsive firefly luciferase and constitutively active *Renilla* luciferase constructs. Cells were treated with increasing concentration of GDF15 (1a) or Activin A control (1b) diluted in DMEM with 0.5 % of FCS, in triplicate. Cells were treated for 6h and luciferase activity measured by luminescence. Firefly luciferase readings are normalised with *Renilla* readings and results plotted to define EC_{50} for the growth factor.