80 Tennis Court Road
Cambridge CB2 1GA, UK
E: orders@qkine.com
T: +44 (0) 1223 491486

QK001_ActA: Human Activin A

Description

Mature domain of human activin A (residues 311-426, Uniprot: P08476) expressed in *E.coli*, refolded and purified to homogeneity. The mature protein is a disulphide linked dimer with a molecular weight of ca. 25 kDa.

Protein is provided in lyophilised form without carrier protein.

Sequence:

GLECDGKVNI CCKKQFFVSF KDIGWNDWII APSGYHANYC EGECPSHIAG
TSGSSLSFHS TVINHYRMRG HSPFANLKSC CVPTKLRPMS MLYYDDGQNI
IKKDIQNMIV EECGCS*

Calculated molecular weight: 25933.58 Da (for the disulphide linked dimer)
Calculated Molecular weight: 12976.03 Da (for protomer in reduced state)
Extinction coefficient at 280 nm: 18950 cm^{-1} mol^{-1} (for protomer)
1.446 cm^{-1} mg^{-1}

Handling instructions:

Activins are very poorly soluble and sticky, so we would recommend following the instructions below closely.

Physiological buffers will cause *massive* precipitation of stock solutions, hence we advise storing the recombinant protein at very low pH to ensure solubility before dilution in cell culture media. Low pH will also assist in maintaining the correct disulphide structure of the protein by minimising disulphide bond exchange reactions.

We recommend dissolving activin A in 10 mM HCl (1:1000 dilution of concentrated HCl) and keep the stock protein concentration at >50 µg/ml to avoid loss by adsorption to plastic. 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. The protein will stand freezing and thawing reasonably well, but as always, it is better to prepare aliquots and stored frozen. If compatible with the end use, add carrier protein (BSA/HSA/gelatin) to activin A to further minimise loss by adsorption. Store in -80°C for long term storage or -20°C for short-term.

The sample is not sterile, and while 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.

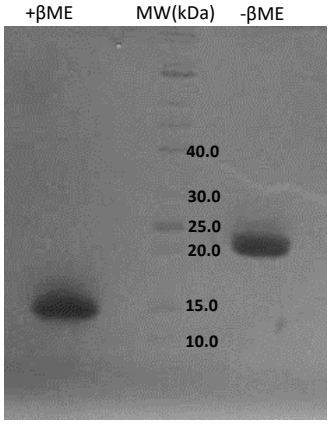
Quality control

Batch: QK001_ActA.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 single band at 25 kDa in non-reducing conditions and is fully reduced into a monomeric species upon reduction. No contaminating protein bands are visible.



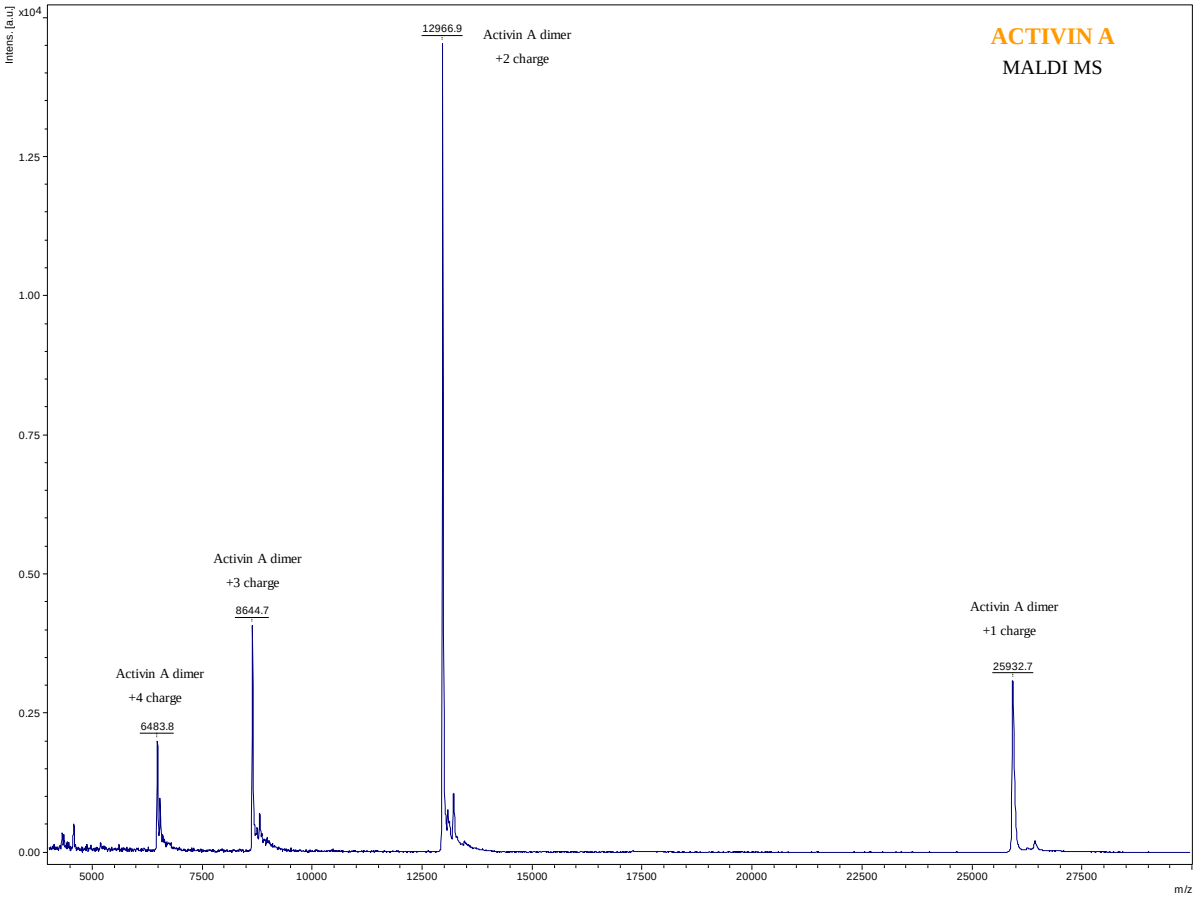
Analysis of the purity of ActA.010 using SDS-PAGE

Activin A.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. Non-reduced Activin A runs as the dimeric species with molecular mass 25 kDa, and when reduced as a single band at 13 kDa.

Purity and identity: mass spectrometry

MALDI mass spectrometric analysis is used to confirm expected molecular mass of the intact protein, with the assumption that all the cysteines are disulphide linked.

Expected MW: 25932.0 Da
Result analysis: 25931.5 Da
Result confirms molecular mass of dimer.

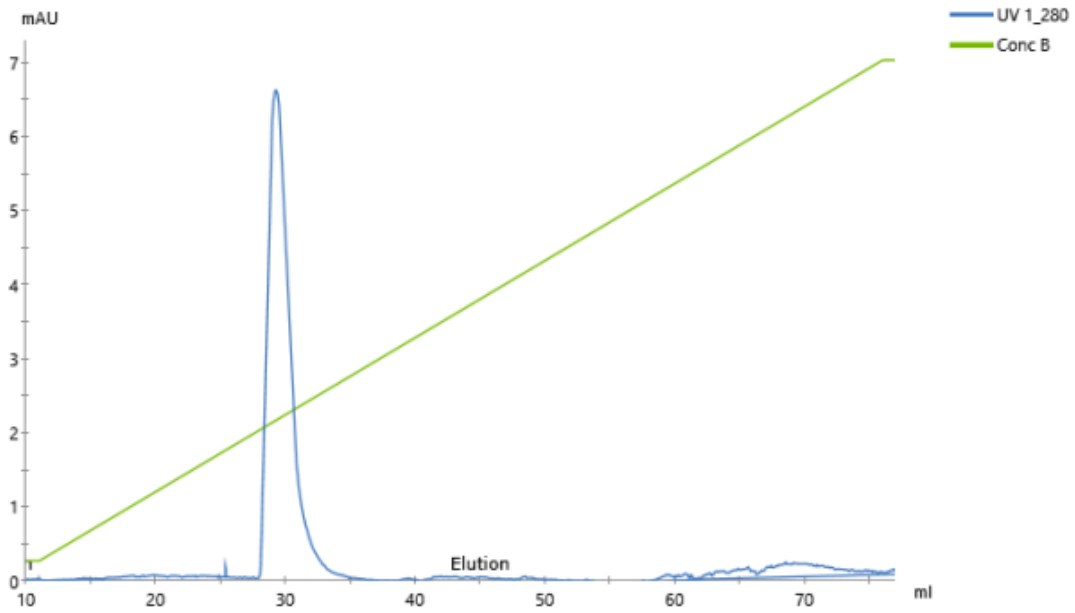


Mass spectrometry analysis of ActA.010 shows protein at expected molecular mass.

ActA.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 of the disulphide-linked dimeric protein.

Purity: analytical reverse phase

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



Reverse phase chromatogram of ActA.010 shows single sharp peak.

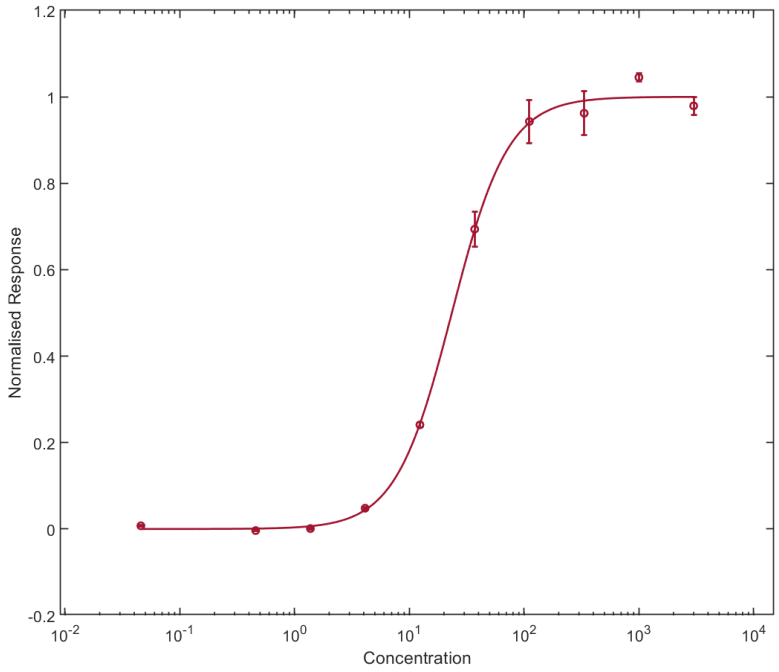
50 µg of Activin A.010 was diluted in 10 mM HCl to 0.1 mg/ml and run in an analytical ACE C4 4.6 x 250 mm column at 1 ml/min and eluted using a 10 – 90 % acetonitrile gradient in 0.1 % trifluoro acetic in 65 minutes.

Blue line shows absorbance at 280 nm and the green line the acetonitrile gradient. Protein eluted in a sharp single peak which confirms that QK001_Activin A.010 has a very high level of purity and homogeneity.

Bioactivity

Bioactivity of Activin A.010 was determined using an activin responsive luciferase reporter in HEK293T cells and compared to a reference control.

Result: EC_{50} = 23.44 pM



Dose response curve for ActA.010 batch confirms EC_{50} is within the desired range.

HEK293T cells transfected with Activin-responsive firefly luciferase and constitutively active *Renilla* luciferase constructs, are treated with increasing concentration of Activin A (diluted in DMEM with 0.5 % of FCS), in triplicate. Cells are grown over-night 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.

Purity – endotoxin testing

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

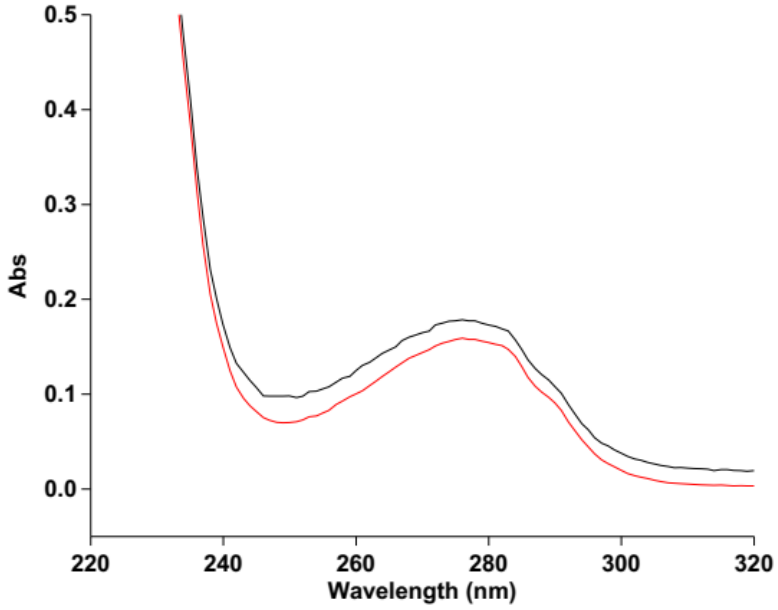
Stem cell cultures are sensitive to endotoxins[1], which can be present in media, serum and as a contaminant on plasticware. We optimise our protein production processes to ensure the lowest possible levels of endotoxin contamination. We set our endotoxin pass criteria at the industry leading <0.1 EU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

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

Recovery

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

Absorbance at 280 nm: average 0.16355
Recovered concentration: 0.16355 cm^{-1} x 10 / 1.446 cm^{-1} mg ml^{-1} = 1.1 mg / ml
Recovery: **110 %** (>100% due to routine 10% over-fill of vials during aliquoting)



UV spectrum of Activin A.010 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 measured in duplicate (orange and black line). Concentration was calculated using extinction coefficient at 280 nm.

References

1. 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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