

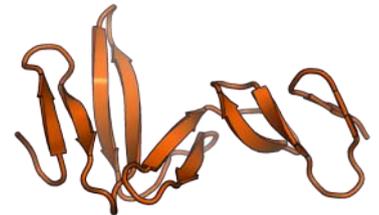
Product datasheet

Qk006 RSPO1: recombinant human R-spondin 1

Summary

Receptor-binding active domain of human R-spondin 1 (Uniprot: [Q2MKA7](#), comprising two furin-like domains expressed in *E. coli*, refolded and purified to homogeneity.

Alternative names: Roof plate-specific spondin-1, Rspo1, R-spondin-1, Cristin 3



Form

Protein is provided in lyophilised form without carrier protein.

Molecular weight

12.5 kDa

Handling guidance

Resuspension in physiological buffers may cause precipitation of stock solutions, hence we recommend dissolving R-spondin 1 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. 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.

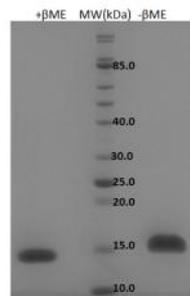
Batch specific quality testing

Qk006 RSPO1 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 Catherine (support@qkine.com).

Purity: SDS-PAGE

The protein migrates as a single band at ca. 16 kDa in non-reducing (- β ME) conditions and 13 kDa upon reduction (+ β ME).



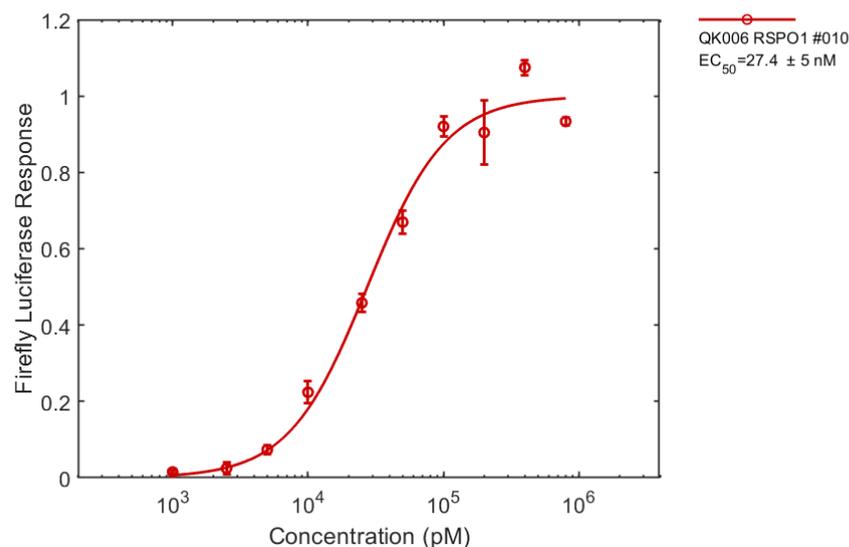
RSPO1 #010 purity confirmed using SDS-PAGE

RSPO1 #010 protein (7 μ g) was resolved using 15% w/v SDS-PAGE in reduced (+ β -mercaptoethanol, + β ME) and non-reduced conditions (- β ME) and stained with Coomassie Brilliant Blue R250.

Bioactivity

Bioactivity of RSPO1 #010 shown using a Wnt reporter assay in HEK293T cells

Result: EC₅₀ = 27 nM



Qk006 RSPO 1 enhances Wnt signalling in TOP-Flash reporter assay

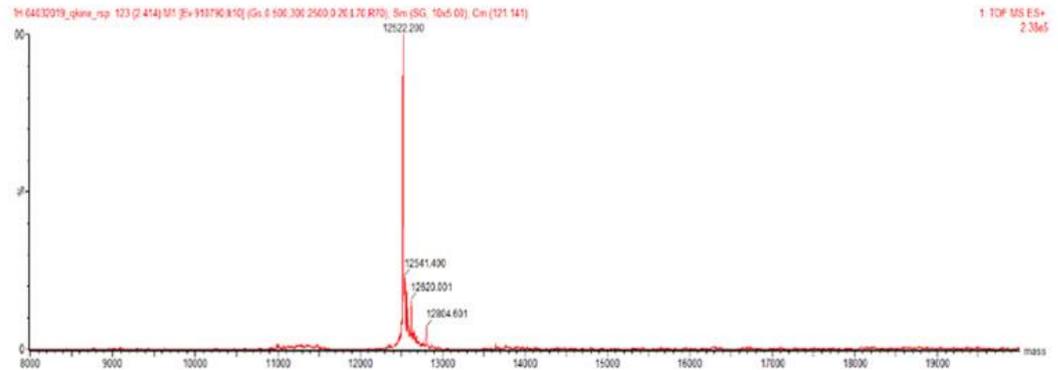
HEK293T cells transfected with Wnt-responsive firefly luciferase reporter TOP-Flash, are treated with increasing concentration of Qk006 R-spondin 1 #010 (diluted in DMEM with 0.5 % of FCS), in the presence of Wnt-conditioned media (1:8 dilution), in triplicate. Cells are grown over-night and luciferase activity measured by luminescence. RSP1 enhances Wnt- β catenin signaling in HEK239T cells.

Batch specific quality testing

Qk006 RSPO1 batch #010

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.



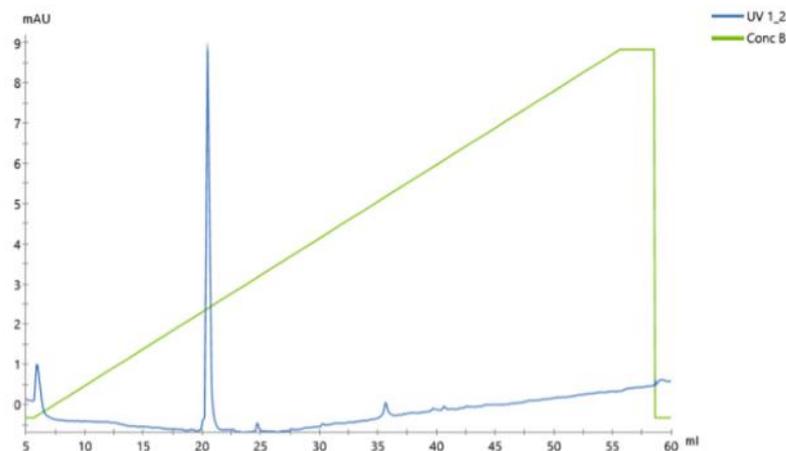
Mass spectrometry analysis of RSPO1 #011 shows protein at expected molecular mass.

RSPO1 #010 in 100 mM sodium phosphate pH 7.4 was analysed by mass spec. 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.

Theoretical molecular weight: **12521 Da**. Result of the analysis: **12522.2 Da** confirming the molecular weight of active domain of R-spondin 1. There are no minor contaminants.

Purity: analytical reverse phase

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



Reverse phase chromatogram of RSPO1 #010 shows single sharp peak.

50 µg of RSPO1 batch #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 acid 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 Qk006 RSPO1 is pure and homogeneous.

Batch specific quality testing

Qkoo6 RSPO1 batch #010

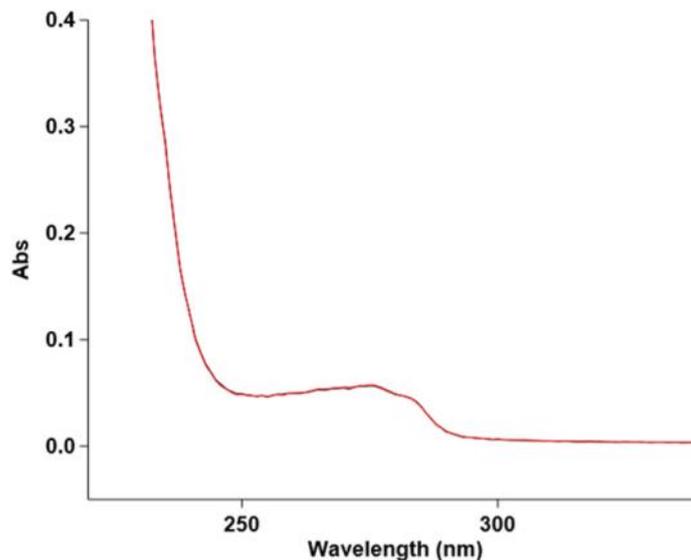
Recovery

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

Absorbance at 280 nm: average 0.05

Recovered concentration: $0.05 \text{ cm}^{-1} \times 10 / 0.436 \text{ cm}^{-1} \text{ mg ml}^{-1} = 1.14 \text{ mg / ml}$

Recovery: 110% (>100% due to routine 10% over-fill of vials during aliquoting)



UV spectrum of RSPO1 batch #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. Concentration was calculated using extinction coefficient at 280 nm.

Endotoxin analysis

Stem cell cultures are sensitive to endotoxins¹, 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.

RSPO1 batch #010 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.

Please note: all our products are for research use only and not for diagnostic or therapeutic use
