Animal-Free™ Recombinant Human R-Spondin 1

Catalog Number: Qk006

DESCRIPTION

Source	*E. coli*-derived human R-Spondin 1 protein Accession # Q2MKA7.1
Predicted Molecular Mass	13 kDa

SPECIFICATIONS

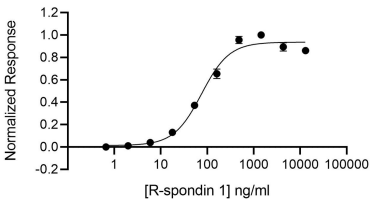
SDS-PAGE	Monomeric R-spondin 1 protein only
Activity	No significant difference between EC_{50} of reference and test lots
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Mass Spectrometry	Single species with expected mass
Mycoplasma	Negative when tested in both ribosomal RNA hybridization and luminescence assays
Formulation	Lyophilized from acetonitrile/TFA See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Resuspend in 10mM HCl at >100 µg/ml, prepare single use aliquots, add carrier protein if desired.
Shipping	The product is shipped lyophilized at ambient temperture, on ice blocks or dry ice. Shipping at ambient temperture does not affect the bioactivity or stability of the protein. Upon reciept, store immediately at the conditions stated below.
Stability & Storage	BulkLotPrefix assignment required for Storage Info

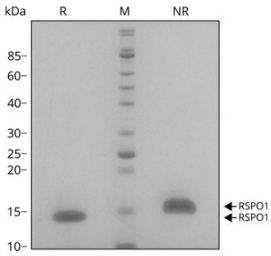
DATA

Bioactivity



Recombinant Human R-spondin 1, Animal-Free Protein Bioactivity R-spondin 1 activity is determined using the Wnt-responsive firefly luciferase reporter assay as it enhances Wnt-ß catenin signaling in HEK239T cells. HEK293T cells transfected with reporter TOP-FLASH are treated in triplicate with increasing concentration of R-spondin 1 (diluted in DMEM with 0.5 % of FCS), in the presence of Wnt-conditioned media (1:8 dilution). Cells are grown overnight, and luciferase activity is measured and normalized. EC_{50} = 75.5 ng/ml (5.8 nM).

SDS-PAGE



Recombinant Human R-spondin 1, Animal-Free Protein SDS-PAGE RSPO1 migrates as a single band at 16 kDa in non-reducing (NR) and 13 kDa in reducing (R) conditions. Purified recombinant protein (7 µg) was resolved using 15% w/v SDS-PAGE in reduced (+β-mercaptothanol, R) and non-reduced conditions (NR) and stained with Coomassie Brilliant Blue R250.

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BACKGROUND

R-Spondin 1 (RSPO1, Roof plate-specific Spondin 1), also known as cysteine-rich and single thrombospondin domain containing protein 3 (Cristin 3), is a 27 kDa secreted protein that shares ~40% amino acid (aa) identity with three other R-Spondin family members (1, 2). All R-Spondins regulate Wnt/ beta-Catenin signaling but have distinct expression patterns (1-3). R-Spondin 1 competes with the Wnt antagonist DKK-1 for binding to the Wnt co-receptors, Kremen and LRP-6, reducing their DKK-1-mediated internalization (4). However, reports are mixed on whether R-Spondin 1 binds LRP-6 directly (4-6). R-Spondin 1 is expressed in early development at the roof plate boundary and is thought to contribute to dorsal neural tube development (3, 7). In humans, rare disruptions of the R-Spondin 1 gene are associated with tendencies for XX sex reversal (phenotypic male) or hermaphroditism, indicating a role for R-Spondin 1 in gender-specific differentiation (7, 8). Mutations in R-Spondin 1 are also linked with palmoplantar keratoderma, abnormal thickening of the skin on the palms of the hands and soles of the feet (7, 8). Postnatally, R-Spondin 1 is expressed by neuroendocrine cells in the intestine, adrenal gland and pancreas, and by epithelia in kidney and prostate (9). Injection of recombinant R-Spondin 1 in mice causes activation of beta-catenin and proliferation of intestinal crypt epithelial cells, and ameliorates experimental colitis (9, 10). Interest in R-Spondin 1 as a cell culture supplement has grown with the expansion of the organoid field. R-Spondin 1 is widely used in organoid cell culture workflows as a vital component that promotes both growth and survival of 3D organoids (11).

Structurally similar to other R-Spondins, R-Spondin 1 contains two adjacent cysteine-rich furin-like domains (aa 34-135) with one potential N-glycosylation site, followed by a thrombospondin (TSP-1) motif (aa 147-207) and a region rich in basic residues (aa 211-263). Only the furin-like domains are needed for beta-catenin stabilization (2, 12). A putative nuclear localization signal at the C-terminus may allow some expression in the nucleus (13). Potential isoforms of 200 and 236 aa have an alternate, shorter N-terminus or are missing aa 146-208, respectively (14). Over aa 21-263, human R-Spondin 1 shares 89%, 87%, 92%, 91%, 91% and 89% aa identity with mouse, rat, horse, dog, goat, and cow RSPO-1, respectively.

References:

1. Chen, J-Z. *et al.* (2002) Mol. Biol. Rep. **29**:287.
2. Kim, K.-A. *et al.* (2006) Cell Cycle **5**:23.
3. Nam, J.-S. *et al.* (2007) Gene Expr. Patterns **7**:306.
4. Binnerts, M.E. *et al.* (2007) Proc. Natl. Acad. Sci. USA **104**:14700.
5. Nam, J.-S. *et al.* (2006) J. Biol. Chem. **281**:13247.
6. Wei, Q. *et al.* (2007) J. Biol. Chem. **282**:15903.
7. Kamata, T. *et a*l. (2004) Biochim. Biophys. Acta **1676**:51.
8. Parma, P. *et al.* (2006) Nat. Genet. **38**:1304.
9. Kim, K.-A. *et al.* (2005) Science **309**:1256.
10. Zhao, J. *et al.* (2007) Gastroenterology **132**:1331.
11. Drost and Clevers. (2018) Nature Reviews Cancer **18**:407.
12. Kazanskaya, O. *et al.* (2004) Dev. Cell **7**:525.
13. Tomaselli, S. *et al.* (2008) Hum. Mutat. **29**:220.
14. UniProt # Q2MKA7.

PRODUCT SPECIFIC NOTICES

The above product was manufactured, tested and released by R&D System's contract manufacturer, Qkine Ltd, at 1 Murdoch House, Cambridge, UK, CB5 8HW. The product is for research use only and not for the diagnostic or theraputic use.

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