

DESCRIPTION

Source *E. coli*-derived human R-Spondin 3 protein
Accession # Q9BXY4.1

Predicted Molecular Mass 17 kDa

SPECIFICATIONS

SDS-PAGE Monomeric R-spondin 3 protein only

Activity No significant difference between EC₅₀ 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

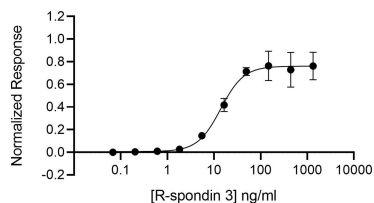
Reconstitution Resuspend in 10 mM HCl at >100 µg/ml, prepare single use aliquots, add carrier protein if desired.

Shipping The product is shipped lyophilized at ambient temperature, on ice blocks or dry ice. Shipping at ambient temperature does not affect the bioactivity or stability of the protein. Upon receipt, store immediately at the conditions stated below.

Stability & Storage Store lyophilized protein between -20 and -80 °C until the date of expiry. Avoid freeze-thaw cycles.

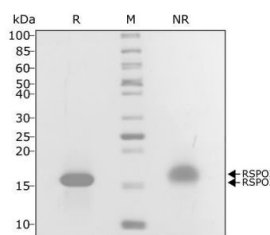
DATA

Bioactivity



Recombinant Human R-spondin 3, Animal-Free Protein Bioactivity Cells transfected with reporter TOP-FLASH are treated in triplicate with increasing concentration of R-spondin 3 in the presence of Wnt-conditioned media (1:8 dilution). RSP1 enhances Wnt-β catenin signaling in HEK239T cells. Cells are grown overnight, and luciferase activity is measured and normalized. EC₅₀ = 14 ng/ml (0.8 nM).

SDS-PAGE



Recombinant Human R-spondin 3, Animal-Free Protein SDS-PAGE R-spondin 3 bioactive domain migrates at 17 kDa in non-reducing (-βME) conditions and upon reduction (+βME). Purified recombinant protein (7 µg) was resolved using 15% w/v SDS-PAGE in reduced (+β-mercaptoethanol, R) and non-reduced conditions (NR) and stained with Coomassie Brilliant Blue R250.

BACKGROUND

R-Spondin 3 (RSPO3, roof plate-specific spondin 3), also called cysteine-rich and single thrombospondin domain containing-1 (Cristin 1), is an ~31 kDa secreted protein that shares ~40% amino acid (aa) identity with the other three R-Spondin family members (1, 2). All are positive modulators of Wnt/ β -catenin signaling, but each has a distinct expression pattern (1-4). Like other R-spondins, R-Spondin 3 contains two adjacent cysteine-rich furin-like domains (aa 35-135) with one potential N-glycosylation site (aa 36), followed by a thrombospondin (TSP-1) motif (aa 147-207) and a region rich in basic residues (aa 211-269). Only the furin-like domains are needed for β -catenin stabilization (2). Within aa 21-209, human R-Spondin 3 shares 93%, 92%, 97%, 96% and 92% aa identity with mouse, rat, equine, bovine and canine R-Spondin 3, respectively. Potential isoforms of 279 and 297 aa diverge at aa 210 and 276, respectively (5). Mouse R-Spondin 3 is critical for development of the placental labyrinthine layer, probably by promoting VEGF expression and thus vascular development (6, 7). It is also essential for expression of the placenta-specific transcription factor, Gcm1. In the mouse embryo, R-Spondin 3 is often expressed by or located near endothelial cells (6). It is found in the roof plate, tail, somites, otic vesicles, cephalic mesoderm, truncus arteriosus, atrioventricular canal of the developing heart, and strongly but transiently in developing limbs (4, 7). R-Spondins regulate Wnt/ β -catenin by competing with the Wnt antagonist DKK-1 for binding to the Wnt co-receptors LRP-6 and Kremen, reducing their DKK-1-mediated internalization (8, 9). Reports differ on whether R-Spondins bind LRP-6 directly (8-10). R-Spondin 3 has also been identified as an oncogene (11).

References:

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2. Kim, K.-A. *et al.* (2008) *Mol. Biol. Cell* **19**:2588.
3. Hendrickx, M. and L. Leys (2008) *Develop. Growth Differ.* **50**:229.
4. Nam, J.-S. *et al.* (2007) *Gene Expr. Patterns* **7**:306.
5. Entrez Accession # EAW48114 and EAW48116.
6. Kazanskaya, O. *et al.* (2008) *Development* **135**:3655.
7. Aoki, M. *et al.* (2007) *Dev. Biol.* **301**:218.
8. Binnerts, M.E. *et al.* (2007) *Proc. Natl. Acad. Sci. USA* **104**:14700.
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10. Wei, Q. *et al.* (2007) *J. Biol. Chem.* **282**:15903.
11. Theodorou, V. *et al.* (2007) *Nat. Genet.* **6**:759.

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 therapeutic use.