

DESCRIPTION

Source Chinese Hamster Ovary cell line, CHO-derived human R-Spondin 3 protein
Gln22-His272
Accession # Q9BXY4

N-terminal Sequence Analysis No results obtained: Gln22 predicted

Predicted Molecular Mass 28.3 kDa

SPECIFICATIONS

SDS-PAGE 36-45 kDa, reducing conditions

Activity Measured by its ability to induce Topflash reporter activity in HEK293T human embryonic kidney cells.
The ED₅₀ for this effect is 0.500-2.00 ng/mL in the presence of 5 ng/mL Recombinant Mouse Wnt-3a (Catalog # 1324-WN).

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution Reconstitute at 100 µg/mL in PBS.

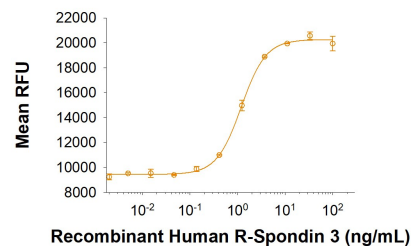
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

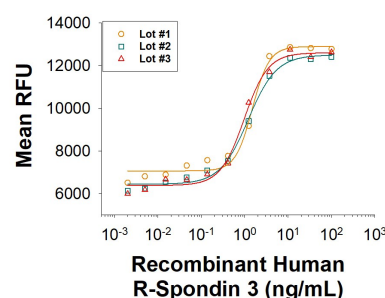
DATA

Bioactivity



Recombinant Human R-Spondin 3 Protein Bioactivity
Recombinant Human R-Spondin 3 (Catalog # 3500-RS/CF), in the presence of Recombinant Mouse Wnt 3a (Catalog # 1324-WN; 5 ng/mL), induces activation of beta-catenin in HEK293T cells measured using the Topflash assay. The ED₅₀ for this effect is 0.5-2.0 ng/mL.

Bioactivity



Recombinant Human R-Spondin 3 Protein, CF Lot-to-Lot Consistency
The lot-to-lot consistency of Recombinant Human R-Spondin 3 (Catalog # 3500-RS/CF) was assessed by testing the ability of three independent lots of the protein to stimulate activation of beta-Catenin using a TOPflash beta-Catenin/TCF reporter assay in the HEK293T human kidney cell line, in the presence of 5 ng/mL Recombinant Mouse Wnt-3a (Catalog # 1324-WN/CF). Each trace on the graph represents data obtained from Recombinant Human R-Spondin 3 from a different manufacturing run. The ED₅₀ for this effect is 0.500-2.00 ng/mL in the presence of 5 ng/mL Recombinant Mouse Wnt-3a.

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:

1. Chen, J.-Z. *et al.* (2002) *Mol. Biol. Rep.* **29**:287.
2. Kim, K.-A. *et al.* (2008) *Mol. Biol. Cell* **19**:2588.
3. Hendrickx, M. and L. Leyns (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.
9. Nam, J.-S. *et al.* (2006) *J. Biol. Chem.* **281**:13247.
10. Wei, Q. *et al.* (2007) *J. Biol. Chem.* **282**:15903.
11. Theodorou, V. *et al.* (2007) *Nat. Genet.* **6**:759.