

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived mouse R-Spondin 3 protein		
	Mouse RSP003 (Gln22, Met33-Gly209) Accession # Q2TJ95.2	IEGRMD	Mouse IgG _{2a} (Glu98-Lys330)
	N-terminus		C-terminus
N-terminal Sequence Analysis	Gln22 (blocked) & Met 33		
Structure / Form	Disulfide-linked homodimer		
Predicted Molecular Mass	48 kDa		

SPECIFICATIONS

SDS-PAGE	54-65 kDa, under reducing conditions
Activity	Measured by its ability to activate TCF reporter activity in HEK293 human embryonic kidney cells in the presence of Wnt-3a. The ED ₅₀ for this effect is 0.300-3.60 ng/mL.
Endotoxin Level	<0.10 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 with Trehalose. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 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. <ul style="list-style-type: none"> • 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

<p>Bioactivity</p> <p>Recombinant Mouse R-Spondin 3 mFc Protein Bioactivity. Recombinant Mouse R-Spondin 3 mFc Protein (Catalog # 11540-RS) activates TCF reporter activity in HEK293 human embryonic kidney cells in the presence of Wnt-3a. The ED₅₀ for this effect is 0.300-3.60 ng/mL.</p>	<p>SDS-PAGE</p> <p>Recombinant Mouse R-Spondin 3 mFc Protein SDS-PAGE. 2 µg/lane of Recombinant Mouse R-Spondin 3 mFc Protein (Catalog # 11540-RS) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 54-65 kDa and 110-130 kDa, respectively.</p>
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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% 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 (amino acids (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, mouse R-Spondin 3 shares 93%, 97%, 96%, 95% and 91% aa identity with human, rat, equine, bovine and canine R-Spondin 3, respectively. Potential isoforms of 217, 224 and 252 aa are divergent or truncated at the C terminus; the 252 aa form also lacks aa 4-33 at the N-terminus (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. Leys (2008) *Develop. Growth Differ.* **50**:229.
4. Nam, J.-S. *et al.* (2007) *Gene Expr. Patterns* **7**:306.
5. Entrez Accession # BAB28811, BAC36296 and EDL04842.
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.