**Recombinant Human R-Spondin 3**  
**Catalog Number: 3500-RS**

### DESCRIPTION

**Source**  
Chinese Hamster Ovary cell line, CHO-derived  
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 $E_{50}$ for this effect is 0.5-2.0 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 with BSA as a carrier protein. See Certificate of Analysis for details.

### PREPARATION AND STORAGE

**Reconstitution**  
Reconstitute at 100 µg/mL in PBS containing at least 0.1% of human or bovine serum albumin.

**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.

### 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:
5. Entrez Accession # EAW48114 and EAW48116.

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