

APM171Hu61 100µg
Active R-Spondin 1 (RSPO1)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Ser21~Ala263

Tags: N-terminal His-tag

Purity: >90%

Traits: Freeze-dried powder

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 5% trehalose.

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 9.4

Predicted Molecular Mass: 28.4kDa

Accurate Molecular Mass: 42kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affect the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 10mM PBS (pH7.6) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

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SRGIKGRQRRIISAEGSQACAKGCELCSEVNGCLKCSPKLFILLERNDIRQVGVCLPSCPPGYFDARNPDMNCKICKCKIEHCEACFSHN  
FCTKCKEGLYLHKGRCPACPEGSSAANGTMECSSPAQCEMSEWSPWGPCSKKQQLCGFRRGSEERTRRVLHAPVGDHAACSDTKETRR  
CTVRRVPCPEGQRRKGGQGRRENANRNLARKESKEAGSRRRKGQQQQQGGTVGPLTSAGPA
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[ACTIVITY]

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. 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. Postnatally, R-Spondin 1 is expressed by neuroendocrine cells in the intestine, adrenal gland and pancreas, and by epithelia in kidney and prostate. Besides, Leucine Rich Repeat Containing G Protein Coupled Receptor 5 (LGR5) has been identified as an interactor of RSPO1, thus a binding ELISA assay was conducted to detect the interaction of recombinant human RSPO1 and recombinant mouse LGR5. Briefly, biotin-linked RSPO1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to LGR5-coated microtiter

wells and incubated for 1h at 37 °C . Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C . Finally, add 50 µl stop solution to the wells and read at 450nm immediately. The binding activity of recombinant human RSPO1 and recombinant mouse LGR5 was shown in Figure 1, the EC50 for this effect is 0.099 ug/mL.

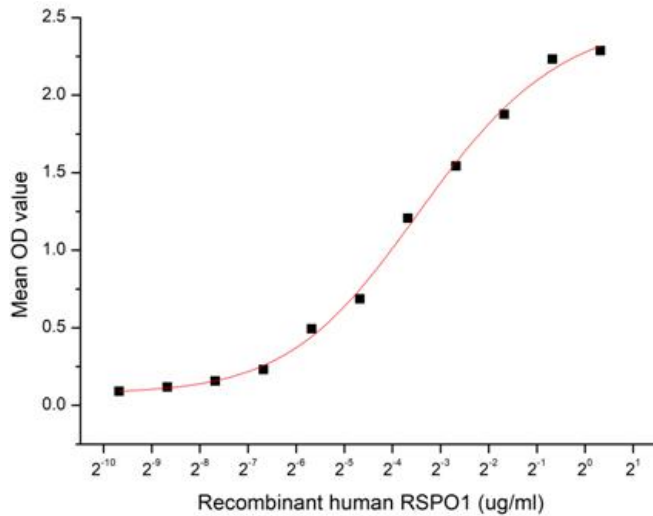


Figure 1. The binding activity of recombinant human RSPO1 and recombinant mouse LGR5

[IDENTIFICATION]

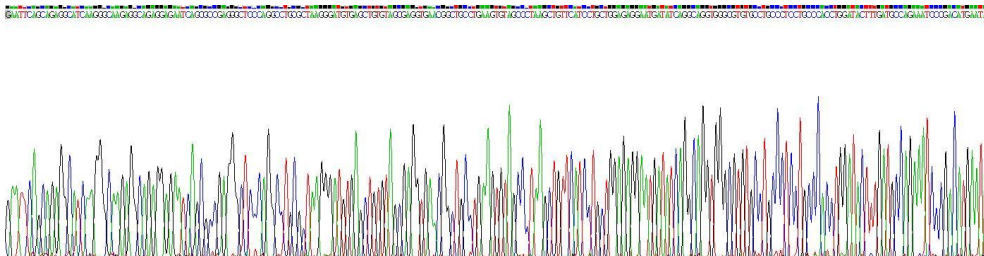


Figure 2. Gene Sequencing (extract)

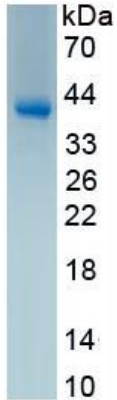


Figure 3. SDS-PAGE

Sample: Active recombinant RSPO1, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.