APB504Hu01 500µg Active Tumor Necrosis Factor Receptor Superfamily, Member 1B (TNFRSF1B) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Ala298~Leu441

Tags: Two N-terminal Tags, His-tag and GST-tag

Purity: >95%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 4.9

Predicted Molecular Mass: 44.8kDa

Accurate Molecular Mass: 50kDa 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 affects 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.

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[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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.

[<u>SEQUENCE</u>]

AKV

PHLPADKARG TQGPEQQHLL ITAPSSSSSS LESSASALDR RAPTRNQPQA PGVEASGAGE ARASTGSSDS SPGGHGTQVN VTCIVNVCSS SDHSSQCSSQ ASSTMGDTDS SPSESPKDEQ VPFSKEECAF RSQLETPETL L

[ACTIVITY]

Tumor necrosis factor receptor superfamily member 1B (TNFRSF1B), also known as tumor necrosis factor receptor 2 (TNFR2) and CD120b, is a membrane receptor that binds tumor necrosis factor-alpha (TNF α). This protein and TNF-receptor 1 form a heterocomplex that mediates the recruitment of two anti-apoptotic proteins, c-IAP1 and c-IAP2, which possess E3 ubiquitin ligase activity. Besides, Tumor Necrosis Factor Alpha (TNF α) has been identified as an interactor of TNFRSF1B, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human TNFRSF1B and recombinant human TNFA. Briefly, TNFRSF1B were diluted serially in PBS with 0.01% BSA

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(pH 7.4). Duplicate samples of 100 μ I were then transferred to TNFa-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-TNFRSF1B pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 μ L stop solution to the wells and read at 450nm immediately. The binding activity of TNFRSF1B and TNFa was shown in Figure 1, the EC50 was 0.0470~0.085 ug/ml.

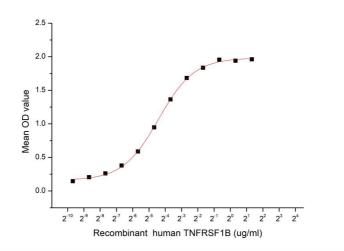
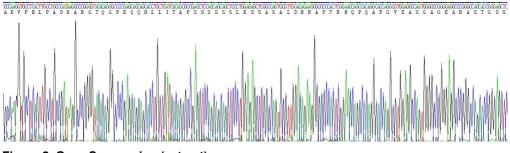
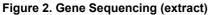


Figure 1. The binding activity of TNFRSF1B with TNFa

[IDENTIFICATION]





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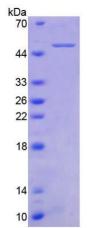


Figure 3. SDS-PAGE

Sample: Active recombinant TNFRSF1B, Human

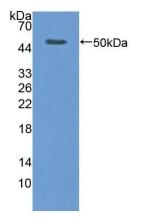


Figure 4. Western Blot

Sample: Recombinant TNFRSF1B, Human;

Antibody: Rabbit Anti-Human TNFRSF1B Ab (PAB504Hu01)

[<u>IMPORTANT NOTE</u>]

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.