

APK300Hu62 100µg
Active RAB7A, Member RAS Oncogene Family (RAB7A)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Thr2~Cys207

Tags: N-terminal His-tag

Purity: >95%

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: 6.6

Predicted Molecular Mass: 25.0kDa

Accurate Molecular Mass: 30kDa 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.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) 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]

TSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQIWDTA
GQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPFVVLGNKIDL
ENRQVATKRAQAWCYSKNNIPYFETSACEAINVEQAFQTIARNALKQETEVLYNEFPEPIKLDK
NDRAKASAESCSC

[ACTIVITY]

RAB7A is a ubiquitous small GTPase, which controls transport to late endocytic compartments. Silencing or overexpression of wild type RAB7A changed the soluble/insoluble rate of peripherin indicating that RAB7A is important for peripherin organization and function. Besides, disease-causing RAB7A mutant proteins bind more strongly to peripherin and their expression causes a significant increase in the amount of soluble peripherin. The altered interaction between disease-causing RAB7A mutants and peripherin could play an important role in CMT2B neuropathy. The activity of recombinant human RAB7A was measured by its ability to hydrolyze the substrate GTP to phosphate which was detected by the malachite green phosphate detection Kit (Beyotime # S0196M). The reaction was performed in the assay buffer 20 mM tris, 200 mM NaCl and 10% glycerol, pH 7.5,

initiated by addition 100 μ L of various concentrations of RAB7A (diluted by assay buffer) to 100 μL of 50 uM GTP. The final well serves as a negative control with no RAB7A, replaced with 100 μ L assay buffer. Incubated at 37°C for 5min, then add 70 ul phosphate detection agent and incubated at room temperature for 30min, read at a wavelength of 630 nm. The specific activity of recombinant human RAB7A is > 800 pmol/min/μg.

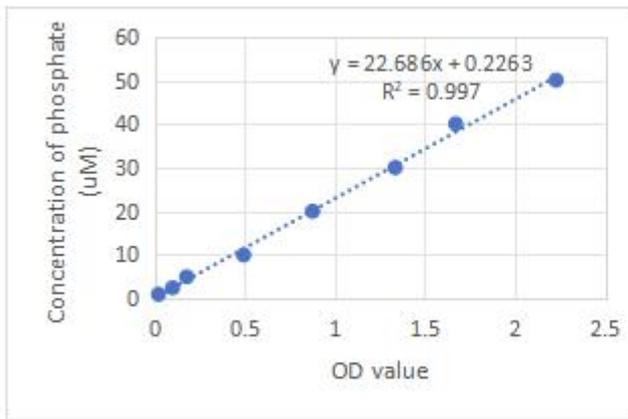


Figure 1. The standard curve of Phosphate

OD(630nm)	Phosphate (uM)
0.0216	1
0.1005	2.5
0.1783	5
0.4947	10
0.8771	20
1.3354	30
1.6711	40
2.2282	50

[IDENTIFICATION]

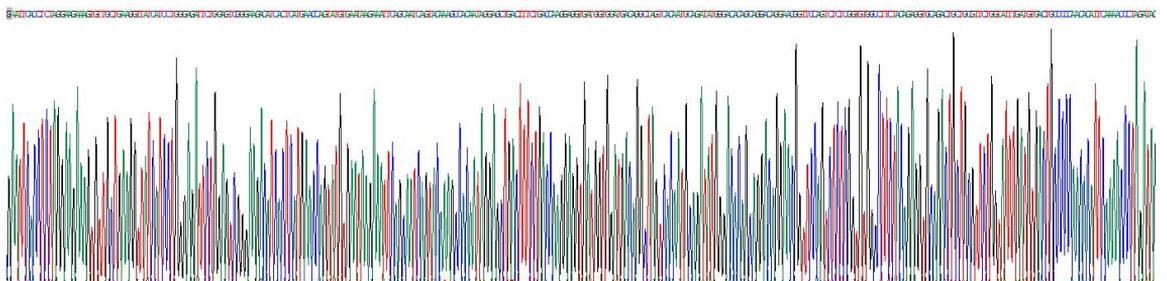


Figure 2. Gene Sequencing (extract)

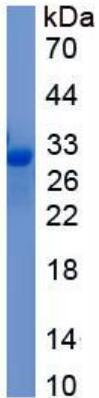


Figure 3. SDS-PAGE

Sample: Active recombinant RAB7A, 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.