

# anti-PIKFYVE-RAB-S1



## Applications

Competition ELISA	Western Blot	SPR	Spiked IP	Immunofluorescence	IP-MS	ChIP
Pass			Pass	Pass		

\*rAb has been tested for the following applications. See below for the experimental details.

## Antibody information

**rAb ID:** anti-PIKFYVE-RAB-S1

**Description:** recombinant Fab fragment obtained by recombinant antibody (rAb) phage display recognizing *PIKFYVE* protein under non-denaturing conditions; specificity and affinity tested.

**Binder type:** rAb **Isotype:** IgG1 **Species:** *Homo sapiens* **Produced in:** *E. coli* **rAb tags:** no tag; Flag-tag

**Specificity:** reacts with *Homo sapiens* PIKFYVE **Epitope:** binds to folded domain amino acids 342-488

**Storage conditions:** short term – store at 4°C (over 6 months), long term - PBS -20°C or -80°C

**Link:** <http://recombinant-antibodies.org/binders/anti-PIKFYVE-RAB-S1>

## Antigen information

**Protein Name:** 1-phosphatidylinositol 3-phosphate 5-kinase

**HGNC Symbol:** PIKFYVE **HGNC ID:** 23785 **Species:** *Homo sapiens*

**UniProt AC:** Q9Y2I7 **UniProt KB:** FYV1\_HUMAN

**Protein Sequence:**

MSGLNDIFEAQKIEWHEHHHHHHENLYFQSHMTEDERKILLDSVQLKDLWKKICHSSGMEFQDHRYWLRTHPNCIVGKE  
LVNWLIRNGHIATRAQAIAIGQAMVDGRWLDCVSHHDQLFRDEYALYRPLQSTEFSETPSPDSDSVNSVEGHSEPSWFKDI  
KFDDSDTEQIAEEGDDNL

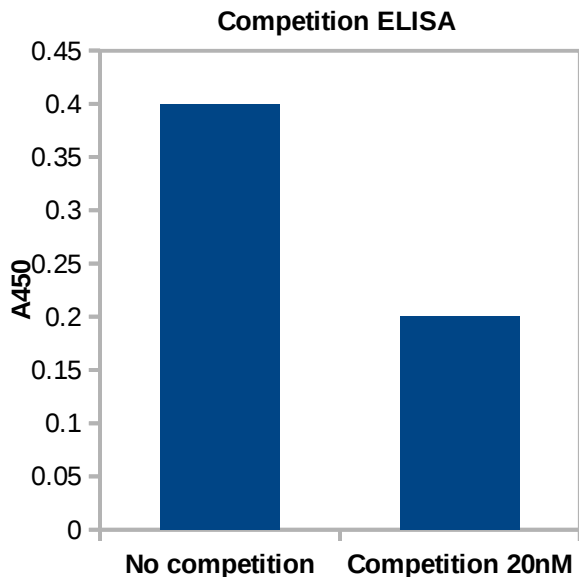
**Tag N-terminus:** MSGLNDIFEAQKIEWHEHHHHHHENLYFQSHM **Tag C-terminus:**

**Vector Type:** pET15Avi6HT\_NESG **Vector Link:** [http://beta.labgeni.us/registries/DNASU/pET15Avi6HT\\_NESG/](http://beta.labgeni.us/registries/DNASU/pET15Avi6HT_NESG/)

**Protein Sequence Position:** 342-488 **Antigen source:** *E. coli* **Source Lab:** Rutgers **Source Lab ID:** HR7108D.015

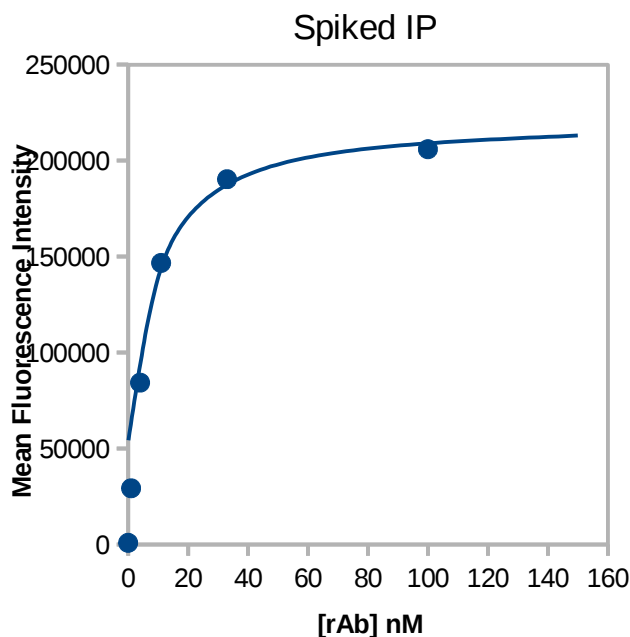
**Description:** affinity purified recombinant protein

## Validation data



**Single point competition phase ELISA** Plot represents specific binding of a target to the rAb-phage in solution (right bar) in comparison to binding to the target immobilized on the plate surface (left bar). Experimental conditions were calibrated to capture binders with dissociation Constant ( $K_D$ ): 20nM or lower.

**Experimental Conditions:** Culture supernatants containing rAb-phage were diluted five-fold in phosphate-buffered saline, 0.5% (w/v) BSA, 0.1% (v/v) Tween 20 either with or without soluble antigen competitor at 20 nM. After 1 h incubation at room temperature, the mixtures were transferred to neutravidin coated plates preloaded with 50  $\mu$ L of 20 nM biotinylated antigen and incubated for 15 min. The plates were washed with phosphate-buffered saline, 0.05% (v/v) Tween 20 and incubated for 30 min with horse radish peroxidase/anti-M13 antibody conjugate (1:5000 dilution). The plates were washed, developed with 3,3',5,5'-Tetramethyl-benzidine/ $H_2O_2$  peroxidase substrate (Thermo Scientific), quenched with 1M  $H_3PO_4$ , and the absorbance at 450 nm (A450) was determined.



**Spiked IP** Tritration curve of rAb against antigen of interest. The  $K_D$  values were obtained by the least-squares fitting of fluorescence saturation data.

**Dissociation Constant ( $K_D$ ):**  $6.2 \pm 0.6$  nM

**Experimental Conditions:**

**Spiked IP:** Antigen was immobilized to M280 Dynabeads. A rAb, 50 nM, was pulled down from high salt AFC buffer with or without HEK293 lysate (OD280 ~10). Beads were washed with low salt AFC buffer, and the captured antibody was quantified with an anti-Fab fluorophore labeled antibody on a flow cytometer.

**Affinity Measurement:** Antigen was immobilized to M280 Dynabeads and incubated with a rAb, in varying concentration (100 nM down to 1 nM in three-fold dilutions). Beads were washed with BSET/BSA and quantified.

**Buffers:**

High salt AFC buffer: 10 mM Tris-HCl, pH 7.9, 420 mM NaCl, 0.1% NP-40

Low salt AFC buffer: 10 mM Tris-HCl, pH 7.9, 100 mM NaCl, 0.1% NP-40

PBSE/BSA: 20 mM  $Na_2HPO_4$ , pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% BSA

PBSET/BSA: PBSE/BSA + 0.1% Tween-20

**Immunofluorescence:**

**Status:** Pass

**Experimental Conditions:** <http://recombinant-antibodies.org/protocols/immunofluorescence>

**IP-MS – immunoprecipitation for mass spectrometric analysis:**

**Status:**

**Experimental Conditions:** <http://recombinant-antibodies.org/protocols/IP-MS>

**ChIP – chromatin immunoprecipitation:**

**Status:**

**Experimental Conditions:** Pending

**IP – immunoprecipitation:**

**Status:**

**Experimental Conditions:** Pending

**SP Elisa:**

**Status:**

**Experimental Conditions:** <http://recombinant-antibodies.org/protocols/ELISA-IC50-EC50-direct-coating>

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