

anti-PIKFYVE-RAB-S5



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-S5

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-S5>

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
LVNWLIRNGHIATRAQAIAIGQAMVDGRWLDVSHHDQLFRDEYALYRPLQSTEFSETPSPDSDSVNSVEGHSEPSWFKDI
KFDDSDTEQIAEEGDDNL

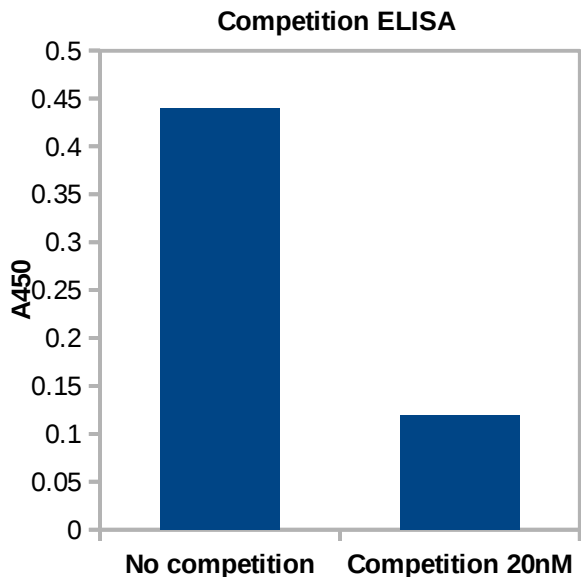
Tag N-terminus: MSGLNDIFEAQKIEWHEHHHHHHENLYFQSHM **Tag C-terminus:**

Vector Type: pET15Avi6HT_NESG **Vector Link:** 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

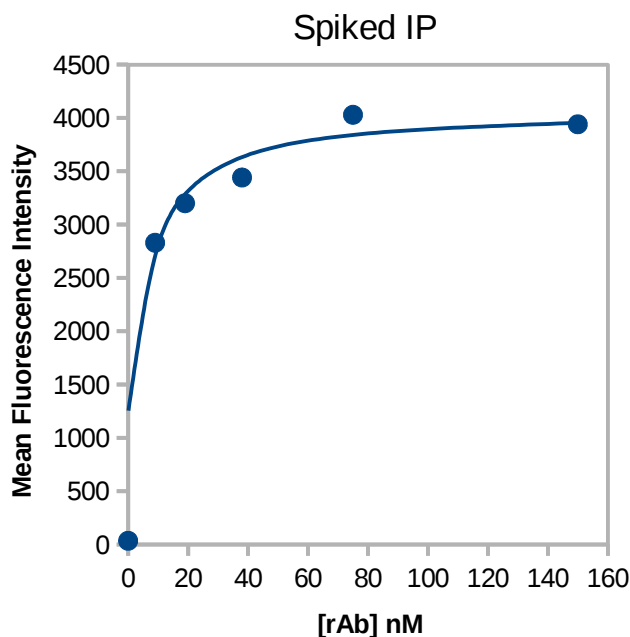
Description: affinity purified recombinant protein

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 μ 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): < 9.4 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 Na₂HPO₄, 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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