

anti-SIRT5-RAB-C176



Applications

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

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

Antibody information

rAb ID: anti-SIRT5-RAB-C176

Description: recombinant Fab fragment obtained by recombinant antibody (rAb) phage display recognizing *SIRT5* protein under non-denaturing conditions; specificity and affinity tested.

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

Specificity: reacts with *Homo sapiens* SIRT5 **Epitope:** binds to folded domain amino acids 34-302

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-SIRT5-RAB-C176>

Antigen information

Protein Name: NAD-dependent protein deacylase sirtuin-5, mitochondrial

HGNC Symbol: SIRT5 **HGNC ID:** 14933 **Species:** *Homo sapiens*

UniProt AC: Q9NXA8 **UniProt KB:** SIR5_HUMAN

Protein Sequence:

MSGLNDIFEAQKIEWHEGSAGGSGARPSSSMADFRKFFAKAKHIVIISGAGVSAESGVPTFRGAGGYWRKWQAQDLATPL
AFAHNPSRVWFEFYHYRREVMGSKPEPNAGHRAIAECETRLGKQGRRVVITQNIDELHRKAGTKNLEIHGSLFKTRCTSC
GVVAENYKSPICPALSGKGAPEPGTQDASIPVEKLPRCEEAGCGLLRPHVWVWFGENLDPAILLEEVDRELAHCDLCLVVG
SSVVYPAAMFAPQVAARGVPVAEFNTETTPATNRFHFHFQGPCGTTLPEALAGGSGHHHHHH

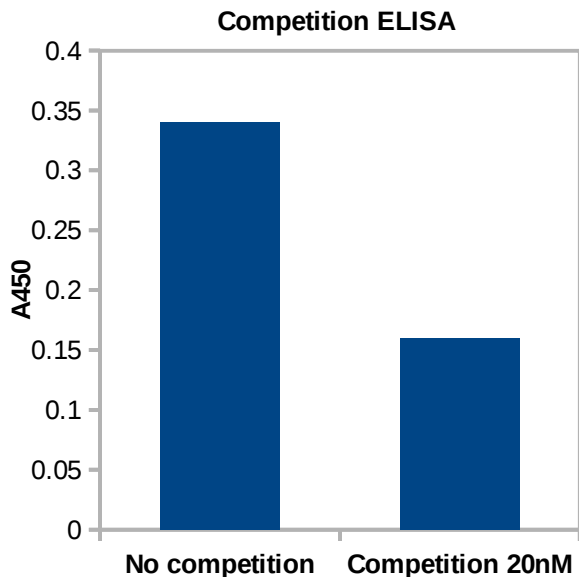
Tag N-terminus: MSGLNDIFEAQKIEWHEGSAGGSG **Tag C-terminus:** GGSGHHHHHH

Vector Type: p28BIOH-LIC **Vector Link:** http://www.thesgc.org/sites/default/files/toronto_vectors/p28BIOH-LIC.pdf

Protein Sequence Position: 34-302 **Antigen source:** *E. coli* **Source Lab:** SGC **Source Lab ID:** SIRT5-A001

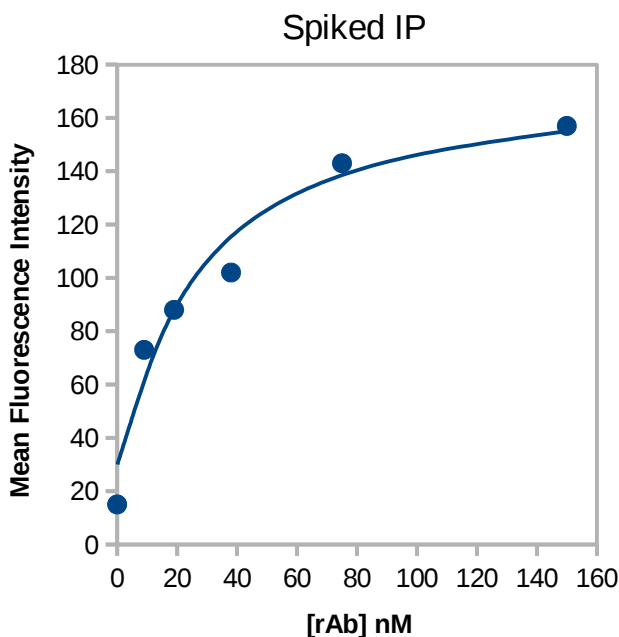
Description: affinity purified recombinant protein

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): 24.1 ± 8.2 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: Pass

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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