anti-SIRT5-RAB-C179

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-C179

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 n 4ºC (over 6 months), long term - PBS -20ºC or -80ºC

Link: http://recombinant-antibodies.org/binders/anti-SIRT5-RAB-C179

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
AFAHNPSRVWEFYHYRREVMGSKEPNAGHRAIAECETRLGKQGRRVVVITQNIDELHRKAGTKNLLEIHGSLFKTRCTSC
GVVAENYKSPICPALSGKGAPEPGTQDASIPVEKLPRCEEAGCGGLLRPHVVWFGENLDPAILEEVDRELAHCDLCLVVGT
SSVVYPAAMFAPQVAARGVPVAEFNTETTPATNRFRFHFQGPCGTTLPEALAGGSGHHHHHH

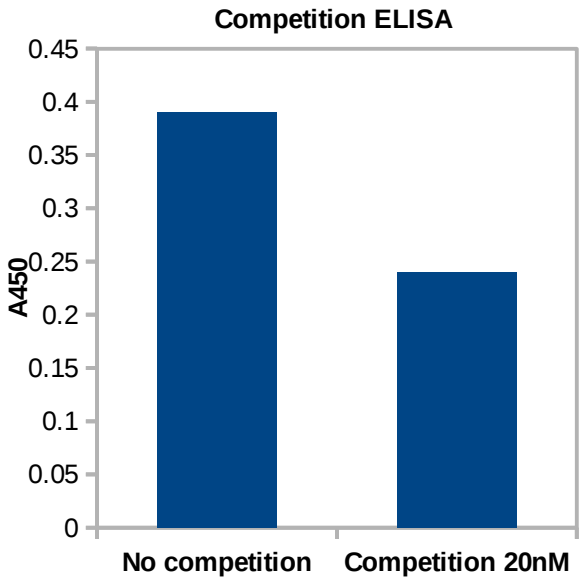
Tag N-terminus: MSGLNDIFEAQKIEWHEGSAGGSG **Tag C-terminus:** GGSGHHHHHH

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

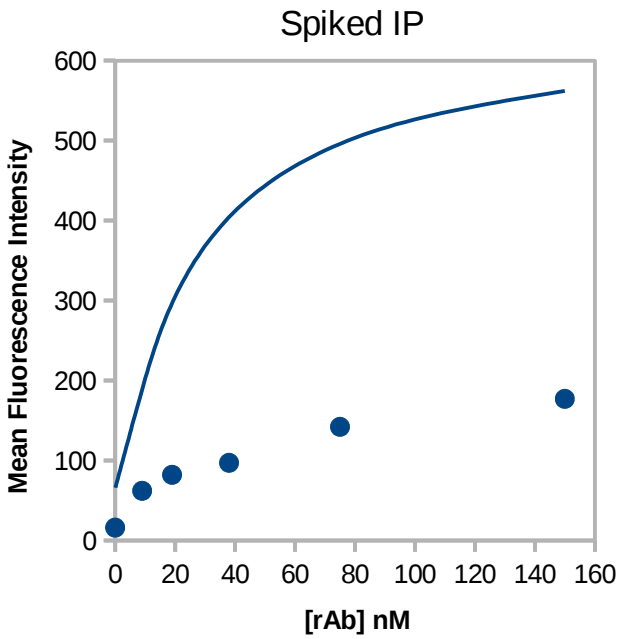
Description: affinity purified recombinant protein

Validation data



Single point competition phage 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 µL 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): 52.9 ± 8.8 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: 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

Visit us at http://recombinant-antibodies.org/

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