

# anti-SIRT5-RAB-C175



## 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-C175

**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-C175>

## 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  
AFAHNPSRVWFEFYHYRREVMGSKEPNAGHRAIAECETRLGKQGRRVVITQNIDELHRKAGTKNLEIHGSLFKTRCTSC  
GVVAENYKSPICPALSGKGAPEPGTQDASIPVEKLPRCEEAGCGLLRPHVWVWFGENLDPAILLEEVDRELAHCDLCLVVG  
SSVVYPAAMFAPQVAARGVPVAEFNTETTPATNRFHFHQGPCGTTLPEALAGGSGHHHHHH

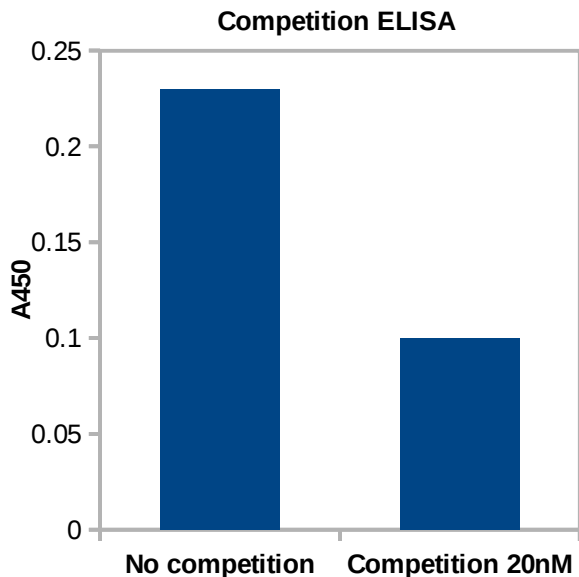
**Tag N-terminus:** MSGLNDIFEAQKIEWHEGSAGGSG **Tag C-terminus:** GGSGHHHHHH

**Vector Type:** p28BIOH-LIC **Vector Link:** [http://www.thesgc.org/sites/default/files/toronto\\_vectors/p28BIOH-LIC.pdf](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

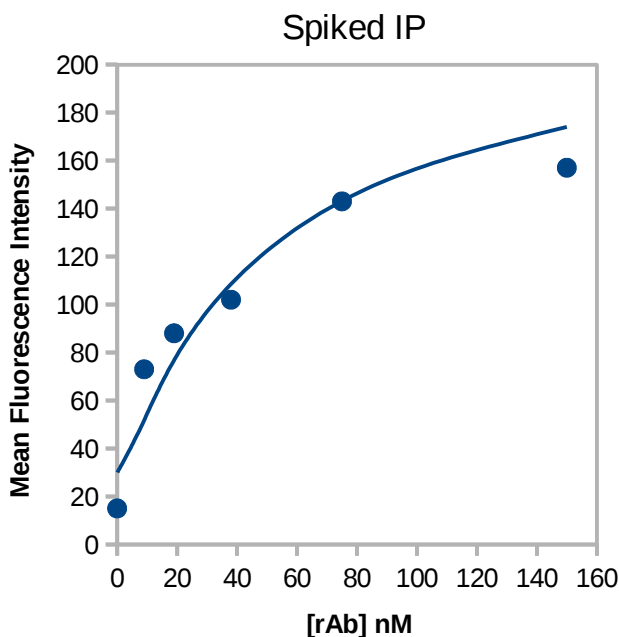
**Description:** affinity purified recombinant protein

## 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  $\mu$ 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 ( $K_D$ ):**  $24.1 \pm 8.2$  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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