# anti-L3MBTL1-RAB-C215



# 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-L3MBTL1-RAB-C215

**Description:** recombinant Fab fragment obtained by recombinant antibody (rAb) phage display recognizing *L3MBTL1* 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* L3MBTL1 Epitope: binds to folded domain amino acids 200-530 Storage conditions: short term – store at n 4°C (over 6 months), long term - PBS -20°C or -80°C Link: http://recombinant-antibodies.org/binders/anti-L3MBTL1-RAB-C215

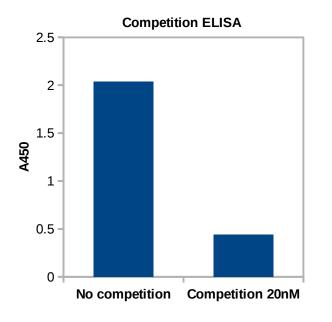
### **Antigen information**

Protein Name: Lethal 3 malignant brain tumor-like protein 1 HGNC Symbol: L3MBTL1 HGNC ID: 15905 Species: Homo sapiens UniProt AC: Q9Y468 UniProt KB: LMBL1\_HUMAN Protein Sequence:

### **Protein Sequence:**

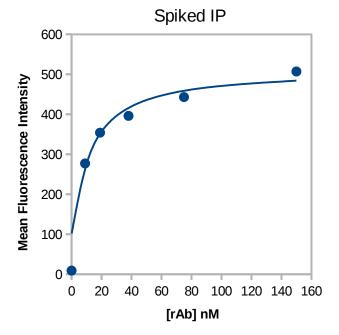
MHHHHHHHHHHDLGTENLYFQSMGEKKECWSWESYLEEQKAITAPVSLFQDSQAVTHNKNGFKLGMKLEGIDPQHPSM YFILTVAEVCGYRLRLHFDGYSECHDFWVNANSPDIHPAGWFEKTGHKLQPPKGYKEEEFSWSQYLRSTRAQAAPKHLFV SQSHSPPPLGFQVGMKLEAVDRMNPSLVCVASVTDVVDSRFLVHFDNWDDTYDYWCDPSSPYIHPVGWCQKQGKPLTP PQDYPDPDNFCWEKYLEETGASAVPTWAFKVRPPHSFLVNMKLEAVDRRNPALIRVASVEDVEDHRIKIHFDGWSHGYDF WIDADHPDIHPAGWCSKTGHPLQPPLGPREPSSASPGGSSKGGYGLNDIFEAQKIEWHE **Tag N-terminus:** MHHHHHHHHHDLGTENLYFQSM **Tag C-terminus:** SSKGGYGLNDIFEAQKIEWHE **Vector Type:** pNIC-Bio2 **Vector Link:** http://www.thesgc.org/sites/default/files/oxford\_vectors/pNIC-Bio2.pdf **Protein Sequence Position:** 200-530 **Antigen source:** *E. coli* **Source Lab:** SGC **Source Lab ID:** L3MBTL1-A003 **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 rAbphage 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 phosphatebuffered 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<sub>2</sub>O<sub>2</sub> peroxidase substrate (Thermo Scientific), guenched 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<sub>D</sub>): < 9.375 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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