Anti-Phospho-Lats2 (Ser83) mAb

Cat# CY-M1020

100 µg (1 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-reactivity	Molecular Wt.	Source Isotype
ST-3B11	**WB, E**	**H**	**125 kDa**	**Mouse IgG2b**

Background: Lats2 is a new member of the Lats tumor suppressor family. The human LATS2 gene is located at chromosome 13q11-12, which has been shown to be a hot spot (67 %) for LOH in nonsmall cell lung cancer.

The Drosophila "large tumor suppressor" (lats) gene encodes a putative serine/threonine kinase. By screening a human testis cDNA library with a mouse LATS2 cDNA, Yabuta et al. (2000) isolated a partial human LATS2 cDNA. The human LATS2 cDNA encodes a deduced 1,046-amino acid partial protein lacking N-terminal sequence. LATS2 contains a PAPA repeat consisting of 7 copies of the dipeptide proline-alanine, which may be involved in protein-protein interactions, and a C-terminal serine/threonine kinase domain. Analysis of the amino acid sequences of the human and mouse LATS2 kinase domains indicated that the LATS2 proteins are most closely related to the LATS1 proteins, followed by Drosophila Lats, among known serine/threonine kinases. Immunoblot analysis of human cell lines showed that endogenous LATS2 is a nuclear protein of approximately 125 kD. Northern blot analysis detected a 5.8-kb LATS2 transcript in several human tissues, with highest expression in heart and skeletal muscle.

Specificity/Sensitivity: Anti-Phospho-Lats2 (Ser83) mAb (ST-3B11) detects phosphorylated recombinant Lats2 only when phosphorylated at serine83, by western blotting.

Source/Purification: Monoclonal antibody is produced by immunizing mice with a synthetic phosphopeptide corresponding to residues surrounding Ser83 of human Lats2. IgG is purified by protein A-sepharose chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 µg/mL, ELISA for detection of Aurora-A kinase activity: 1 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at –20°C.

Applications Key:
WB: Western blotting, **IP:** Immunoprecipitation, **IHC:** Immunohistochemistry, **IC:** Immunocytochemistry, **F:** Flow cytometry, **E:** ELISA, **FP:** Fluorescence polarization assay

Species Cross-Reactivity Key:
H: Human, **M:** Mouse, **R:** Rat, **Hm:** Hamster, **Mk:** Monkey, **Mi:** Mink, **C:** Chicken, **X:** Xenopus, **Z:** Zebra fish (Species enclosed in parentheses are predicted to react based on 100 % sequence homology.)

References:

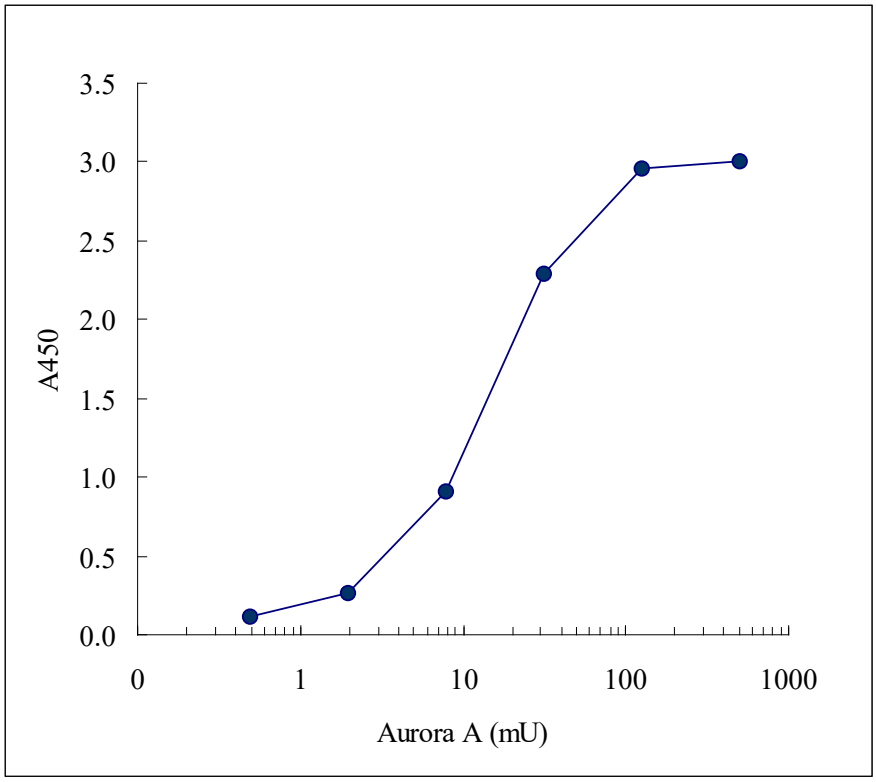
1. Tao, W.; Zhang, S.; Turenchalk, G. S.; Stewart, R. A.; St. John, M. A. R.; Chen, W.; Xu, T. Human homologue of the Drosophila melanogaster lats tumour suppressor modulates CDC2 activity. *Nature Genet*. **21**, 177-181, 1999.

2. Li Y, Pei J, Xia H, Ke H, Wang H, Tao W. Lats2, a putative tumor suppressor, inhibits G1/S transition. *Oncogene*. **22**(28):4398-405, 2003

3. Ishizaki K, Fujimoto J, Kumimoto H, Nishimoto Y, Shimada Y, Shinoda M, Yamamoto T. Frequent polymorphic changes but rare tumor specific mutations of the LATS2 gene on 13q11-12 in esophageal squamous cell carcinoma. *Int J Oncol*. **21**(5):1053-7, 2002

4. Yabuta N, Fujii T, Copeland NG, Gilbert DJ, Jenkins NA, Nishiguchi H, Endo Y, Toji S, Tanaka H, Nishimune Y, Nojima H. Structure, expression, and chromosome mapping of LATS2, a mammalian homologue of the Drosophila tumor suppressor gene lats/warts. *Genomics*. **63**(2):263-70, 2000

Fig.1 ELISA for measurement of recombinant Aurora A kinase activity using Anti-Phospho-Lats2 (Ser83) mAb (ST-3B11)



Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20 % methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2 % w/v SDS, 10 % glycerol, 50 mM DTT, 0.01 % w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1 % Tween-20 with 5 % w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100 %).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1 % Tween-20 with 5 % blocking agent; for 20 mL, add 2 mL 10X TBS to 18 mL water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100 %).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1 % Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm^2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3,000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.

2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten seconds exposure should indicate the proper exposure time.

For more information, please visit our web site**.**
https://ruo.mbl.co.jp/

MANUFACTURED BY

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL: https://ruo.mbl.co.jp
E-mail: support@mbl.co.jp