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antibody and ELISA experts

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Anti-CDC25B Antibody Picoband™

Catalog Number: A01899-1

About CDC25B

Central to the onset of mitosis in all eukaryotic cells is the CDC2 protein kinase, the activity of which is negatively regulated by phosphorylation and positively activated by dephosphorylation. The latter function is carried out by a specific phosphatase, CDC25. At least 3 human CDC25 genes code for the A, B, and C forms of CDC25. CDC25B is mapped to 20p13. P38 kinase has a critical role in the initiation of a G2 delay after ultraviolet radiation. Inhibition of p38 blocks the rapid initiation of this checkpoint in both human and murine cells after ultraviolet radiation. In vitro, p38 binds and phosphorylates CDC25B at serines 309 and 361, and CDC25C at serine-216; phosphorylation of these residues is required for binding to 14-3-3 proteins. In vivo, inhibition of p38 prevents both phosphorylation of CDC25B at serine-309 and 14-3-3 binding after ultraviolet radiation, and mutation of this site is sufficient to inhibit the checkpoint initiation. Regulation of CDC25B phosphorylation by p38 is a critical event for initiating the G2/M checkpoint after ultraviolet radiation.

Overview

Product Name	Anti-CDC25B Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-CDC25B Antibody Picoband™ catalog # A01899-1. Tested in Flow Cytometry, ELISA, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na2HPO4.
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	P30305

Technical Details

Immunogen	E.coli-derived human CDC25B recombinant protein (Position: M1-H486).
Predicted Reactive Species	Human
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG

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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.25-0.5 ug/ml, Human, Mouse, Rat Flow Cytometry, 1-3 ug/1x10^6 cells, Human Direct ELISA, 0.1-0.5 ug/ml, Human

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Anti-CDC25B Antibody Picoband™ (A01899-1) Images

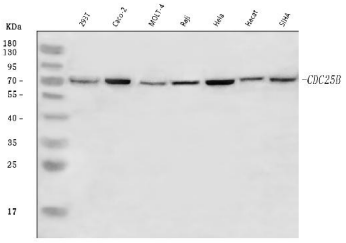

Figure 1. Western blot analysis of CDC25B using anti-CDC25B antibody (A01899-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human 293T whole cell lysates,
Lane 2: human CACO-2 whole cell lysates,
Lane 3: human MOLT-4 whole cell lysates,
Lane 4: human Raji whole cell lysates,
Lane 5: human Hela whole cell lysates,
Lane 6: human Hacat whole cell lysates.
Lane 7: human SiHa whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CDC25B antigen affinity purified polyclonal antibody (Catalog # A01899-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CDC25B at approximately 70 kDa. The expected band size for CDC25B is at 65 kDa.

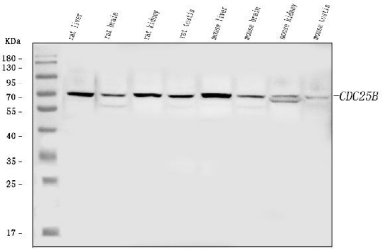

Figure 2. Western blot analysis of CDC25B using anti-CDC25B antibody (A01899-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: rat liver tissue lysates,
Lane 2: rat brain tissue lysates,
Lane 3: rat kidney tissue lysates,
Lane 4: rat testis tissue lysates,
Lane 5: mouse liver tissue lysates,
Lane 6: mouse brain tissue lysates,
Lane 7: mouse kidney tissue lysates,
Lane 8: mouse testis tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CDC25B antigen affinity purified polyclonal antibody (Catalog # A01899-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CDC25B at

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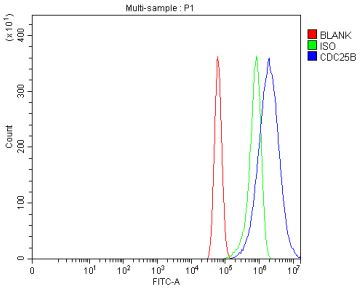

Figure 3. Flow Cytometry analysis of U87 cells using anti-CDC25B antibody (A01899-1).
Overlay histogram showing U87 cells stained with A01899-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CDC25B Antibody (A01899-1, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.