Human/Mouse/Rat CDC25B Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1649

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat CDC25B. Four splice variants of CDC25B, with molecular weights of 61, 63, 65, and 67 kDa, are known. The immunogen selected for this antibody is common to all variants. Immunoreactivity consistent with at least 3 variants has been detected in Western blots. In Western blots, this antibody does not cross-react with recombinant human (rh) CDC25A or rhCDC25C.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human CDC25B Glu391-Gln580 Accession # P30305
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

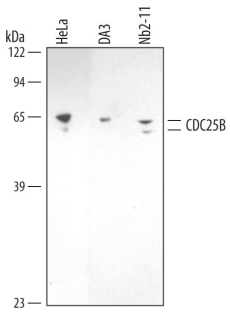
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.3 µg/mL	See Below
Immunocytochemistry	10-25 µg/mL	See Below

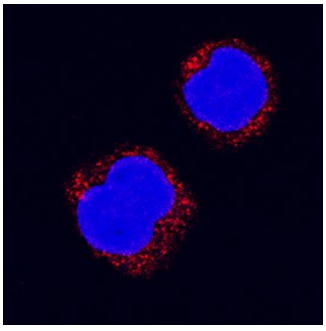
DATA

Western Blot



Detection of Human/Mouse/Rat CDC25B by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, DA3 mouse myeloma cell line, and Nb2-11 rat lymphoma cell line. PVDF membrane was probed with 0.3 µg/mL of Goat Anti-Human/Mouse/Rat CDC25B Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1649) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for CDC25B at approximately 61 - 67 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



CDC25B in HL-60 Human Cell Line. CDC25B was detected in immersion fixed HL-60 human acute promyelocytic leukemia cell line using Goat Anti-Human/Mouse/Rat CDC25B Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1649) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Cell Division Cycle 25B (Cdc25B) phosphatase removes inorganic phosphate groups covalently attached to tyrosine, serine and threonine residues in proteins (1). Breast cancer patients bearing tumors containing high levels of Cdc25B have been found to have a greater incidence of aggressive, high-grade tumors than those with low Cdc25B levels (2). In cells, the levels of Cdc25B activity are highest during the G2/M transition of the cell cycle, where it is suspected to be involved in “checkpoint” control of cell cycle progression (3). Overexpression of Cdc25B reduces the G2/M cell cycle block caused by ionizing radiation (4). Although activated by phosphorylation, Ser323 phosphorylation causes the enzyme to bind the protein 14-3-3, preventing substrate access to the catalytic site (5). One of the major substrates of Cdc25B is Cdc2, a kinase that is activated by dephosphorylation (6). The recombinant protein is truncated to remove the N-terminal regulatory domains and is fully active.

References:

1. Draetta, G. and J. Eckstein (1997) Biochim. Biophys. Acta **1332**:M53.
2. Galaktionov, K. *et al.* (1995) Science **269**:1575.
3. Lammer, C. *et al.* (1998) J. Cell Sci. **111**:2445.
4. Miyata, H. *et al.* (2001) Cancer Res. **61**:3188.
5. Forrest, A. and B. Gabrielli (2001) Oncogene **20**:4393.
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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449