

Anti-CDK1 Antibody Picoband[™] (monoclonal, 2G11)

Catalog # ABO14867

Anti-CDK1 Antibody Picoband[™] (monoclonal, 2G11) - Product Information

Application Primary Accession Host Isotype Reactivity Clonality Format **Description** Anti-CDK1 Antibody F WB, IHC, FC <u>P06493</u> Mouse Mouse IgG2b Rat, Human, Mouse Monoclonal Lyophilized

Anti-CDK1 Antibody Picoband[™] (monoclonal, 2G11) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution Add 0.2ml of distilled water will yield a concentration of 500 μ g/ml.

Anti-CDK1 Antibody Picoband[™] (monoclonal, 2G11) - Additional Information

Gene ID 983

Other Names

Cyclin-dependent kinase 1, CDK1, 2.7.11.22, 2.7.11.23, Cell division control protein 2 homolog, Cell division protein kinase 1, p34 protein kinase, CDK1, CDC2, CDC28A, CDKN1, P34CDC2

Calculated MW 34 kDa KDa

Application Details Western blot, 0.1-0.5 μg/ml
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 μg/ml
 Flow Cytometry, 1-3 μg/1x10^6 cells

Subcellular Localization Nucleus. Mitochondrion. Centrosome. Spindle. Cytoplasm.

Tissue Specificity Isoform 2 is found in breast cancer tissues.

Contents Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

Immunogen E.coli-derived human CDK1 recombinant protein (Position: L66-M297). Human CDK1 shares 97.8% and 98.3% amino acid (aa) sequence identity with mouse and rat CDK1, respectively.

Cross Reactivity



No cross-reactivity with other proteins.

Storage

Store 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 freeze-thaw cycles.

Anti-CDK1 Antibody Picoband[™] (monoclonal, 2G11) - Protein Information

Name CDK1

Synonyms CDC2, CDC28A, CDKN1, P34CDC2

Function

Plays a key role in the control of the eukaryotic cell cycle by modulating the centrosome cycle as well as mitotic onset; promotes G2-M transition via association with multiple interphase cyclins (PubMed:16407259, PubMed: 16933150, PubMed: 17459720, PubMed:18356527, PubMed:19509060, PubMed: 19917720, PubMed:20171170, PubMed:20935635, PubMed:20937773, PubMed: 21063390, PubMed:2188730, PubMed: 23355470, PubMed:2344612, PubMed:23601106, PubMed:23602554, PubMed:25556658, PubMed: 26829474, PubMed: 27814491, PubMed:30139873, PubMed:30704899). Phosphorylates PARVA/actopaxin, APC, AMPH, APC, BARD1, Bcl-xL/BCL2L1, BRCA2, CALD1, CASP8, CDC7, CDC20, CDC25A, CDC25C, CC2D1A, CENPA, CSNK2 proteins/CKII, FZR1/CDH1, CDK7, CEBPB, CHAMP1, DMD/dystrophin, EEF1 proteins/EF-1, EZH2, KIF11/EG5, EGFR, FANCG, FOS, GFAP, GOLGA2/GM130, GRASP1, UBE2A/hHR6A, HIST1H1 proteins/histone H1, HMGA1, HIVEP3/KRC, KAT5, LMNA, LMNB, LBR, MKI67, LATS1, MAP1B, MAP4, MARCKS, MCM2, MCM4, MKLP1, MLST8, MYB, NEFH, NFIC, NPC/nuclear pore complex, PITPNM1/NIR2, NPM1, NCL, NUCKS1, NPM1/numatrin, ORC1, PRKAR2A, EEF1E1/p18, EIF3F/p47, p53/TP53, NONO/p54NRB, PAPOLA, PLEC/plectin, RB1, TPPP, UL40/R2, RAB4A, RAP1GAP, RBBP8/CtIP, RCC1, RPS6KB1/S6K1, KHDRBS1/SAM68, ESPL1, SKI, BIRC5/survivin, STIP1, TEX14, beta-tubulins, MAPT/TAU, NEDD1, VIM/vimentin, TK1, FOXO1, RUNX1/AML1, SAMHD1, SIRT2, CGAS and RUNX2 (PubMed: 16407259, PubMed:16933150, PubMed:17459720, PubMed:18356527, PubMed:19202191, PubMed:19509060, PubMed:19917720, PubMed:20171170, PubMed:20935635, PubMed:20937773, PubMed:21063390, PubMed:2188730, PubMed:23355470, PubMed:2344612, PubMed:23601106, PubMed:23602554, PubMed:25012651, PubMed:25556658, PubMed:26829474, PubMed:27814491, PubMed:30704899, PubMed:32351706, PubMed:34741373). CDK1/CDC2-cyclin-B controls pronuclear union in interphase fertilized eggs (PubMed:18480403, PubMed:20360007). Essential for early stages of embryonic development (PubMed:18480403, PubMed:20360007). During G2 and early mitosis, CDC25A/B/C-mediated dephosphorylation activates CDK1/cyclin complexes which phosphorylate several substrates that trigger at least centrosome separation, Golgi dynamics, nuclear envelope breakdown and chromosome condensation (PubMed:18480403, PubMed:20360007, PubMed:2188730, PubMed:2344612, PubMed:30139873). Once chromosomes are condensed and aligned at the metaphase plate, CDK1 activity is switched off by WEE1- and PKMYT1-mediated phosphorylation to allow sister chromatid separation, chromosome decondensation, reformation of the nuclear envelope and cytokinesis (PubMed:18480403, PubMed:20360007). Phosphorylates KRT5 during prometaphase and metaphase (By similarity). Inactivated by PKR/EIF2AK2- and WEE1-mediated phosphorylation upon DNA damage to stop cell cycle and genome replication at the G2 checkpoint thus facilitating DNA repair (PubMed:20360007). Reactivated after successful DNA repair through WIP1-dependent signaling leading to CDC25A/B/C-mediated dephosphorylation and restoring cell cycle progression (PubMed:20395957). Catalyzes lamin (LMNA, LMNB1 and LMNB2) phosphorylation at the onset of mitosis, promoting nuclear envelope breakdown (PubMed: 2188730, PubMed:2344612, PubMed:37788673). In proliferating cells, CDK1-mediated FOXO1 phosphorylation at the G2-M phase represses FOXO1 interaction with 14-3-3 proteins and thereby promotes FOXO1 nuclear accumulation and transcription factor activity, leading to cell death of postmitotic neurons (PubMed:18356527). The phosphorylation of beta-tubulins regulates microtubule dynamics during mitosis (PubMed:16371510). NEDD1 phosphorylation promotes PLK1-mediated NEDD1 phosphorylation and subsequent targeting of the gamma-tubulin ring complex (gTuRC) to the centrosome, an important step for spindle formation (PubMed:19509060). In addition, CC2D1A phosphorylation regulates CC2D1A spindle pole localization and association with SCC1/RAD21 and centriole cohesion during mitosis (PubMed: 20171170). The phosphorylation of Bcl-xL/BCL2L1 after prolongated G2 arrest upon DNA damage triggers



apoptosis (PubMed:19917720). In contrast, CASP8 phosphorylation during mitosis prevents its

activation by proteolysis and subsequent apoptosis (PubMed:20937773). This phosphorylation occurs in cancer cell lines, as well as in primary breast tissues and lymphocytes (PubMed:20937773). EZH2 phosphorylation promotes H3K27me3 maintenance and epigenetic gene silencing (PubMed:20935635). CALD1 phosphorylation promotes Schwann cell migration during peripheral nerve regeneration (By similarity). CDK1-cyclin-B complex phosphorylates NCKAP5L and mediates its dissociation from centrosomes during mitosis (PubMed: 26549230). Regulates the amplitude of the cyclic expression of the core clock gene BMAL1 by phosphorylating its transcriptional repressor NR1D1, and this phosphorylation is necessary for SCF(FBXW7)- mediated ubiguitination and proteasomal degradation of NR1D1 (PubMed:27238018). Phosphorylates EML3 at 'Thr-881' which is essential for its interaction with HAUS augmin-like complex and TUBG1 (PubMed:30723163). Phosphorylates CGAS during mitosis, leading to its inhibition, thereby preventing CGAS activation by self DNA during mitosis (PubMed:32351706). Phosphorylates SKA3 on multiple sites during mitosis which promotes SKA3 binding to the NDC80 complex and anchoring of the SKA complex to kinetochores, to enable stable attachment of mitotic spindle microtubules to kinetochores (PubMed:28479321, PubMed:31804178, PubMed:32491969).

Cellular Location

Nucleus {ECO:0000250|UniProtKB:P11440}. Cytoplasm {ECO:0000250|UniProtKB:P11440}. Mitochondrion. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Cytoplasm, cytoskeleton, spindle. Note=Cytoplasmic during the interphase Colocalizes with SIRT2 on centrosome during prophase and on splindle fibers during metaphase of the mitotic cell cycle. Reversibly translocated from cytoplasm to nucleus when phosphorylated before G2-M transition when associated with cyclin-B1. Accumulates in mitochondria in G2-arrested cells upon DNA-damage

Tissue Location

[Isoform 2]: Found in breast cancer tissues.

Anti-CDK1 Antibody Picoband[™] (monoclonal, 2G11) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

Anti-CDK1 Antibody Picoband™ (monoclonal, 2G11) - Images





Figure 1. Western blot analysis of CDK1 using anti-CDK1 antibody (M00209-6).

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 50ug of sample under reducing conditions.

Lane 1: human HEK293 whole cell lysates

- Lane 2: human A549 whole cell lysates
- Lane 3: human HepG2 whole cell lysates
- Lane 4: human THP-1 whole cell lysates
- Lane 5: human PANC-1 whole cell lysates
- Lane 6: human SW620 whole cell lysates
- Lane 7: rat RH35 whole cell lysates

Lane 8: mouse NIH/3T3 whole cell lysates

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-CDK1 antigen affinity purified monoclonal antibody (Catalog # M00209-6) at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for CDK1 at approximately 34KD. The expected band size for CDK1 is at 34KD.



Figure 2. IHC analysis of CDK1 using anti-CDK1 antibody (M00209-6).

CDK1 was detected in paraffin-embedded section of human colon cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti-CDK1 Antibody (M00209-6) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was



developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



Figure 3. IHC analysis of CDK1 using anti-CDK1 antibody (M00209-6).

CDK1 was detected in paraffin-embedded section of human tonsil tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti-CDK1 Antibody (M00209-6) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



Figure 4. IHC analysis of CDK1 using anti-CDK1 antibody (M00209-6).

CDK1 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti-CDK1 Antibody (M00209-6) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.





Figure 5. IHC analysis of CDK1 using anti-CDK1 antibody (M00209-6).

CDK1 was detected in paraffin-embedded section of mouse testis tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti-CDK1 Antibody (M00209-6) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



Figure 6. IHC analysis of CDK1 using anti-CDK1 antibody (M00209-6).

CDK1 was detected in paraffin-embedded section of rat testis tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti-CDK1 Antibody (M00209-6) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.





Figure 7. Flow Cytometry analysis of PC-3 cells using anti-CDK1 antibody (M00209-6).

Overlay histogram showing PC-3 cells stained with M00209-6 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CDK1 Antibody (M00209-6,1 μ g/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 μ g/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 μ g/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



Figure 8. Flow Cytometry analysis of U20S cells using anti-CDK1 antibody (M00209-6). Overlay histogram showing U20S cells stained with M00209-6 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CDK1 Antibody (M00209-6, 1 μ g/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 μ g/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 μ g/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-CDK1 Antibody Picoband[™] (monoclonal, 2G11) - Background

CDC2, Cell Division Cycle 2, is also known as CDK1 (Cyclin-dependent Kinase 1). CDC2 is a catalytic subunit of a protein kinase complex, called the M-phase promoting factor that induces entry into mitosis and is universal among eukaryotes. In HeLa cells CDC2 is the most abundant phosphotyrosine-containing protein and its phosphotyrosine content is subject to cell cycle regulation. CDC2 gene is located on chromosome 10.