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Anti-Cdk2 Antibody Picoband™ (monoclonal, 5B12D1)

Catalog # ABO16246

Product Information

Application	WB, IHC, FC
Primary Accession	P24941
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Cdk2 Antibody Picoband™ (monoclonal, 5B12D1) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	1017
Other Names	Cyclin-dependent kinase 2, 2.7.11.22, Cell division protein kinase 2, p33 protein kinase, CDK2, CDKN2
Calculated MW	33930
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Mouse
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 5B12D1
Immunogen	E.coli-derived human Cdk2 recombinant protein (Position: E81-L298). Human Cdk2 shares 98.6% amino acid (aa) sequence identity with rat Cdk2.
Purification	Immunogen affinity purified.
Storage	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.

Protein Information

Name	CDK2
Synonyms	CDKN2
Function	Serine/threonine-protein kinase involved in the control of the cell cycle; essential for meiosis, but dispensable for mitosis (PubMed:10499802, PubMed:10884347, PubMed:10995386, PubMed:10995387, PubMed:11051553, PubMed:11113184, PubMed:12944431, PubMed:15800615, PubMed:17495531, PubMed:19966300, PubMed:20935635, PubMed:21262353, PubMed:21596315, PubMed:28216226, PubMed:28666995). Phosphorylates CABLES1, CTNNB1, CDK2AP2, ERCC6, NBN, USP37, p53/TP53, NPM1, CDK7, RB1, BRCA2, MYC, NPAT, SUV39H1, EZH2 (PubMed:10499802, PubMed:10995386, PubMed:10995387, PubMed:11051553, PubMed:11113184, PubMed:12944431, PubMed:15800615, PubMed:19966300, PubMed:20935635, PubMed:21262353, PubMed:21596315, PubMed:24728993, PubMed:28216226). Triggers duplication of centrosomes and DNA (PubMed:11051553). Acts at the G1-S transition to promote the E2F transcriptional program and the initiation of DNA synthesis, and modulates G2 progression; controls the timing of entry into mitosis/meiosis by controlling the subsequent activation of cyclin B/CDK1 by phosphorylation, and coordinates the activation of cyclin B/CDK1 at the centrosome and in the nucleus (PubMed:18372919, PubMed:19238148, PubMed:19561645). Crucial role in orchestrating a fine balance between cellular proliferation, cell death, and DNA repair in embryonic stem cells (ESCs) (PubMed:18372919, PubMed:19238148, PubMed:19561645). Activated by the CDK-activating kinase (CAK) complex consisting of CDK7, cyclin-H/CCNH and MAT1, which is a master regulator of CDK activity (PubMed:41100585). Activity of CDK2 is maximal during S phase and G2; activated by interaction with cyclin E during the early stages of DNA synthesis to permit G1-S transition, and subsequently activated by cyclin A2 (cyclin A1 in germ cells) during the late stages of DNA replication to drive the transition from S phase to mitosis, the G2 phase (PubMed:18372919, PubMed:19238148, PubMed:19561645). EZH2 phosphorylation promotes H3K27me3 maintenance and epigenetic gene silencing (PubMed:20935635). Cyclin E/CDK2 prevents oxidative stress-mediated Ras-induced senescence by phosphorylating MYC (PubMed:19966300). Involved in G1-S phase DNA damage checkpoint that prevents cells with damaged DNA from initiating mitosis; regulates homologous recombination-dependent repair by phosphorylating BRCA2, this phosphorylation is low in S phase when recombination is active, but increases as cells progress towards mitosis (PubMed:15800615, PubMed:20195506, PubMed:21319273). In response to DNA damage, double-strand break repair by homologous recombination a reduction of CDK2-mediated BRCA2 phosphorylation (PubMed:15800615). Involved in regulation of telomere repair by mediating phosphorylation of NBN (PubMed:28216226). Phosphorylation of RB1 disturbs its interaction with E2F1 (PubMed:10499802). NPM1 phosphorylation by cyclin E/CDK2 promotes its dissociates from unduplicated centrosomes, thus initiating centrosome duplication (PubMed:11051553). Cyclin E/CDK2-mediated phosphorylation of NPAT at G1-S transition and until prophase stimulates the NPAT-mediated activation of histone gene transcription during S phase (PubMed:10995386, PubMed:10995387). Required for vitamin D-mediated growth inhibition by being itself inactivated (PubMed:20147522). Involved in the nitric oxide- (NO) mediated signaling in a nitrosylation/activation-dependent manner (PubMed:20079829). USP37 is activated by phosphorylation and thus triggers G1-S transition (PubMed:21596315). CTNNB1 phosphorylation regulates insulin internalization (PubMed:21262353). Phosphorylates FOXP3 and negatively regulates its transcriptional activity and protein stability (By similarity). Phosphorylates ERCC6 which is essential for its chromatin remodeling activity at DNA double-strand breaks (PubMed:29203878). Acts as

a regulator of the phosphatidylinositol 3-kinase/protein kinase B signal transduction by mediating phosphorylation of the C-terminus of protein kinase B (PKB/AKT1 and PKB/AKT2), promoting its activation (PubMed:24670654).

Cellular Location

Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Nucleus, Cajal body. Cytoplasm. Endosome Note=Localized at the centrosomes in late G2 phase after separation of the centrosomes but before the start of prophase. Nuclear-cytoplasmic trafficking is mediated during the inhibition by 1,25-(OH)(2)D(3)

Background

CDK2, Cyclin-Dependent Kinase2, is also known as P33. The CDK2 protein was highly homologous to p34(CDC2) kinase and more significantly homologous to Xenopus Eg1 kinase, suggesting that CDK2 is the human homolog of Eg1. The CDK2 gene is mapped to 12q13, the same region to which the CDK4 gene maps. Human cyclin A binds independently to 2 kinases, p34(cdc2) or p33. In adenovirus-transformed cells, the viral E1A oncoprotein seems to associate with p33/cyclin A but not with p34(cdc2)/cyclin A. The gene for p33 shares 65% sequence identity with p34(cdc2). P33(cdk2) plays a unique role in cell cycle regulation of vertebrate cells.

Images

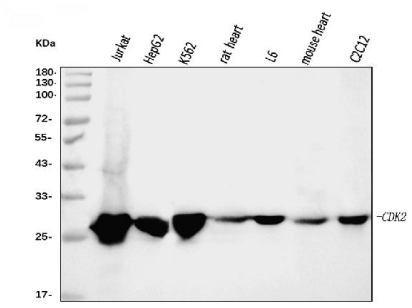
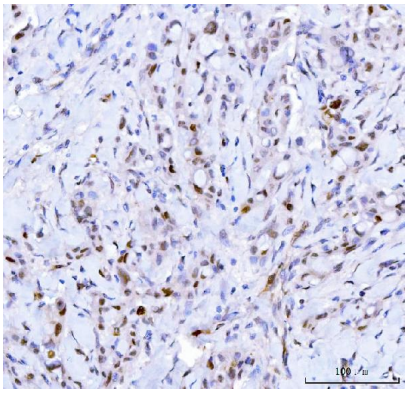


Figure 1. Western blot analysis of Cdk2 using anti-Cdk2 antibody (M00166-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Jurkat whole cell lysates,
Lane 2: human HepG2 whole cell lysates,
Lane 3: human K562 whole cell lysates,
Lane 4: rat heart tissue lysates,
Lane 5: rat L6 whole cell lysates,
Lane 6: mouse heart tissue lysates,
Lane 7: mouse C2C12 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 mouse anti-Cdk2 antigen affinity purified monoclonal antibody (Catalog # M00166-3) at 0.5 µg/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 Cdk2 at approximately 30 kDa. The expected band size for Cdk2 is at 30 kDa.

Figure 2. IHC analysis of Cdk2 using anti-Cdk2 antibody (M00166-3).
Cdk2 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with



10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Cdk2 Antibody (M00166-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

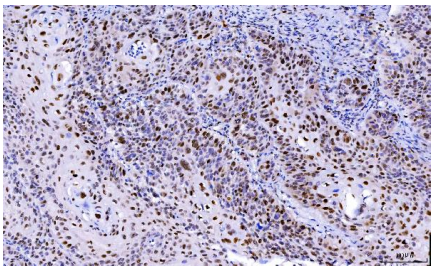


Figure 3. IHC analysis of Cdk2 using anti-Cdk2 antibody (M00166-3).
Cdk2 was detected in a paraffin-embedded section of human laryngeal squamous cell carcinomas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Cdk2 Antibody (M00166-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

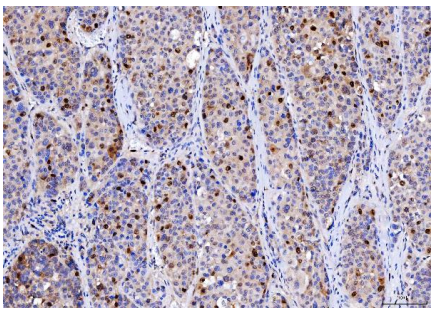


Figure 4. IHC analysis of Cdk2 using anti-Cdk2 antibody (M00166-3).
Cdk2 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Cdk2 Antibody (M00166-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.



Figure 5. Flow Cytometry analysis of ANA-1 cells using anti-Cdk2 antibody (M00166-3).
Overlay histogram showing ANA-1 cells stained with M00166-3 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cdk2 Antibody (M00166-3, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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