

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

Catalog # ABO16246

## Product Information

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

## Additional Information

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

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name</b>     | CDK2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Synonyms</b> | CDKN2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Function</b> | <p>Serine/threonine-protein kinase involved in the control of the cell cycle; essential for meiosis, but dispensable for mitosis (PubMed:<a href="#">10499802</a>, PubMed:<a href="#">10884347</a>, PubMed:<a href="#">10995386</a>, PubMed:<a href="#">10995387</a>, PubMed:<a href="#">11051553</a>, PubMed:<a href="#">11113184</a>, PubMed:<a href="#">12944431</a>, PubMed:<a href="#">15800615</a>, PubMed:<a href="#">17495531</a>, PubMed:<a href="#">19966300</a>, PubMed:<a href="#">20935635</a>, PubMed:<a href="#">21262353</a>, PubMed:<a href="#">21596315</a>, PubMed:<a href="#">28216226</a>, PubMed:<a href="#">28666995</a>). Phosphorylates CABLES1, CTNNB1, CDK2AP2, ERCC6, NBN, USP37, p53/TP53, NPM1, CDK7, RB1, BRCA2, MYC, NPAT, SUV39H1, EZH2 (PubMed:<a href="#">10499802</a>, PubMed:<a href="#">10995386</a>, PubMed:<a href="#">10995387</a>, PubMed:<a href="#">11051553</a>, PubMed:<a href="#">11113184</a>, PubMed:<a href="#">12944431</a>, PubMed:<a href="#">15800615</a>, PubMed:<a href="#">19966300</a>, PubMed:<a href="#">20935635</a>, PubMed:<a href="#">21262353</a>, PubMed:<a href="#">21596315</a>, PubMed:<a href="#">24728993</a>, PubMed:<a href="#">28216226</a>). Triggers duplication of centrosomes and DNA (PubMed:<a href="#">11051553</a>). 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:<a href="#">18372919</a>, PubMed:<a href="#">19238148</a>, PubMed:<a href="#">19561645</a>). Crucial role in orchestrating a fine balance between cellular proliferation, cell death, and DNA repair in embryonic stem cells (ESCs) (PubMed:<a href="#">18372919</a>, PubMed:<a href="#">19238148</a>, PubMed:<a href="#">19561645</a>). 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:<a href="#">41100585</a>). 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:<a href="#">18372919</a>, PubMed:<a href="#">19238148</a>, PubMed:<a href="#">19561645</a>). EZH2 phosphorylation promotes H3K27me3 maintenance and epigenetic gene silencing (PubMed:<a href="#">20935635</a>). Cyclin E/CDK2 prevents oxidative stress-mediated Ras-induced senescence by phosphorylating MYC (PubMed:<a href="#">19966300</a>). 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:<a href="#">15800615</a>, PubMed:<a href="#">20195506</a>, PubMed:<a href="#">21319273</a>). In response to DNA damage, double-strand break repair by homologous recombination a reduction of CDK2-mediated BRCA2 phosphorylation (PubMed:<a href="#">15800615</a>). Involved in regulation of telomere repair by mediating phosphorylation of NBN (PubMed:<a href="#">28216226</a>). Phosphorylation of RB1 disturbs its interaction with E2F1 (PubMed:<a href="#">10499802</a>). NPM1 phosphorylation by cyclin E/CDK2 promotes its dissociates from unduplicated centrosomes, thus initiating centrosome duplication (PubMed:<a href="#">11051553</a>). 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:<a href="#">10995386</a>, PubMed:<a href="#">10995387</a>). Required for vitamin D-mediated growth inhibition by being itself inactivated (PubMed:<a href="#">20147522</a>). Involved in the nitric oxide- (NO) mediated signaling in a nitrosylation/activation-dependent manner (PubMed:<a href="#">20079829</a>). USP37 is activated by phosphorylation and thus triggers G1-S transition (PubMed:<a href="#">21596315</a>). CTNNB1 phosphorylation regulates insulin internalization (PubMed:<a href="#">21262353</a>). 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:<a href="#">29203878</a>). Acts as</p> |

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

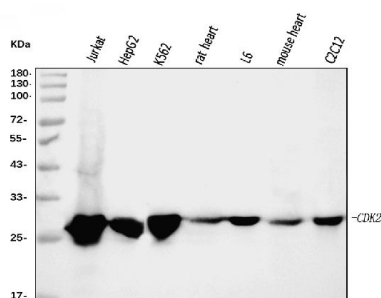


Figure 1. Western blot analysis of Cdk2 using anti-Cdk2 antibody (M00166-3).

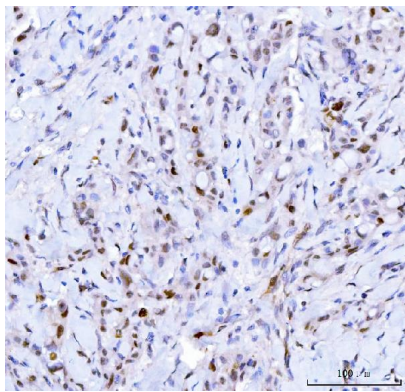
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

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  $\mu\text{g}/\text{ml}$  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.

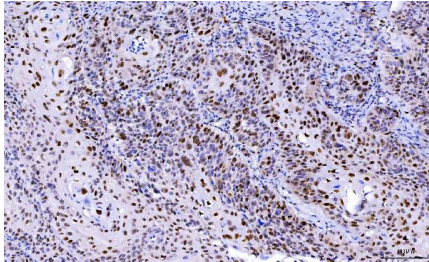


Figure 3. IHC analysis of Cdk2 using anti-Cdk2 antibody (M00166-3). Cdk2 was detected in a paraffin-embedded section of human laryngeal squamous cell carcinomas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu\text{g}/\text{ml}$  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.

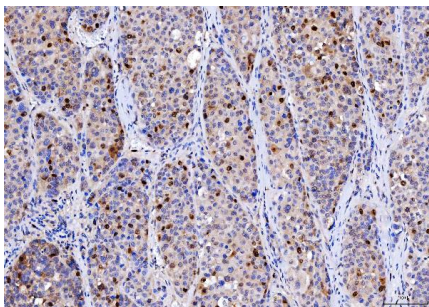


Figure 4. IHC analysis of Cdk2 using anti-Cdk2 antibody (M00166-3). Cdk2 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu\text{g}/\text{ml}$  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.

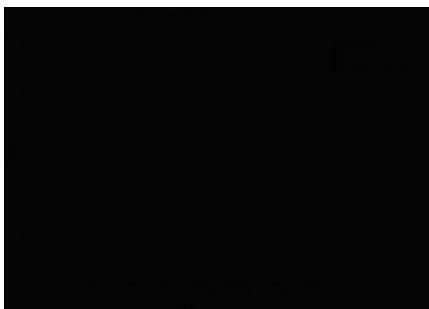


Figure 5. Flow Cytometry analysis of ANA-1 cells using anti-Cdk2 antibody (M00166-3). Overlay histogram showing ANA-1 cells stained with M00166-3 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cdk2 Antibody (M00166-3, 1  $\mu\text{g}/1 \times 10^6$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