Datasheet for ABIN967650

# anti-CDK4 antibody (N-Term)

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## Overview

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| Quantity: | 0.1 mg |
| Target: | CDK4 |
| Binding Specificity: | N-Term |
| Reactivity: | Human, Mouse, Rat, Dog |
| Host: | Mouse |
| Clonality: | Monoclonal |
| Conjugate: | This CDK4 antibody is un-conjugated |
| Application: | Western Blotting (WB), Immunoprecipitation (IP), Immunohistochemistry (Frozen Sections) (IHC (fro)), BioImaging (BI) |

## Product Details

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| Brand: | BD Pharmingen™ |
| Immunogen: | Human Cdk4 |
| Clone: | DCS-35 |
| Isotype: | IgG1 kappa |
| Cross-Reactivity: | Dog (Canine), Rat (Rattus), Mouse (Murine) |
| Characteristics: | 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.<br>2. Please refer to us for technical protocols.<br>3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance. |

## Product Details

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| | 4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.<br>5. Triton is a trademark of the Dow Chemical Company. |
| Purification: | The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. |

## Target Details

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| Target: | CDK4 |
| Alternative Name: | Cdk4 (CDK4 Products) |
| Background: | Cyclins, cyclin-dependent kinases (Cdks), and cyclin-dependent kinase inhibitors (CdkIs) are essential for cell-cycle control in eukaryotes (reviewed in 1). Cyclins, regulatory subunits, bind to cyclin-dependent kinases (Cdks), catalytic subunits, to form active cyclin-Cdk complexes. Cdk subunits by themselves are inactive and binding to a cyclin is required for their activity. Cyclins A, B1, D and E undergo periodic synthesis and degradation, thereby providing a mechanism to regulate Cdk activity throughout the cell cycle. Additionally, Cdk activity is further regulated by activating and inhibitory phosphorylations, and small proteins (p15, p16, p18, p19, p21 and p27), called CdkIs, that bind to cyclins, Cdks, or cyclin-Cdk complexes. Cdk4 was originally called PSK-J3,2 and following demonstration of its association with D-type cyclins, became known as Cdk4.2 D-type cyclins also associate with Cdks 2 and 5, although Cdk4 appears to be the most abundant partner. The D-type cyclins (D1, D2, and D3) are expressed in response to growth factors or mitogens, and rapidly degrade when mitogens are withdrawn. D cyclins appear to promote G0 to G1 transitions and the rate of G1 progression. For example, cyclin D-Cdk4 and cyclin D-Cdk6 complexes phosphorylate the retinoblastoma protein (Rb) which removes the G1 phase block caused by underphosphorylated Rb. Cdk4 has a molecular weight of ~33 kD. |
| Molecular Weight: | 33 kDa |
| Pathways: | Cell Division Cycle, Mitotic G1-G1/S Phases, Regulation of Cell Size |

## Application Details

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| Application Notes: | Bioimaging<br>1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. |

## Application Details

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| | 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT).<br>3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.<br>4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS.<br>5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT.<br>6. Remove the blocking buffer and add 50 myl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.<br>7. Remove the primary antibody, and wash the wells three times with 100 myl of 1× PBS.<br>8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl to each well, and incubate in the dark for 1 hour at RT.<br>9. Remove the second step reagent, and wash the wells three times with 100 myl of 1× PBS.<br>10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging.<br>11. View and analyze the cells on an appropriate imaging instrument. |
| Comment: | Related Products: ABIN967389 |
| Restrictions: | For Research Use only |

## Handling

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| Format: | Liquid |
| Concentration: | 0.5 mg/mL |
| Buffer: | Aqueous buffered solution containing ≤0.09 % sodium azide. |
| Preservative: | Sodium azide |
| Precaution of Use: | This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only. |
| Storage: | 4 °C |
| Storage Comment: | Store undiluted at 4°C. |

## Publications

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| Product cited in: | Johnson, Walker: "Cyclins and cell cycle checkpoints." in: **Annual review of pharmacology and** |

## Publications

**toxicology**, Vol. 39, pp. 295-312, (1999) (PubMed).

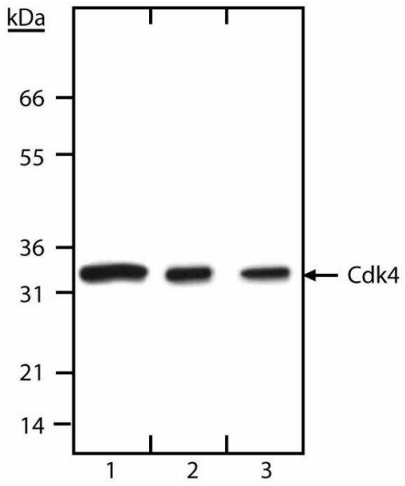
Matsushime, Ewen, Strom, Kato, Hanks, Roussel, Sherr: "Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins." in: **Cell**, Vol. 71, Issue 2, pp. 323-34, (1992) (PubMed).

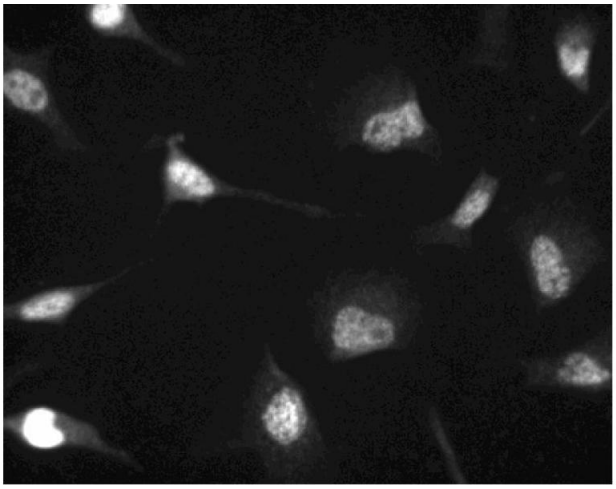
## Images

**Image 1.**


**Western Blotting**

**Image 2.** Western blot analysis of Cdk4. 293 cell lysates were probed with 5 µg/ml (lane 1), 2 µg/ml (lane 2) or 0.5 µg/ml (lane 3) of anti-Cdk4 (clone DCS-35). The antibody identifies Cdk4 at ~33 kDa.





**Immunofluorescence**

**Image 3.** Immunofluorescent staining of HeLa (ATCC CCL-2) cells. Cells were seeded in a 96 well imaging plate at ~10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-Cdk4 antibody. The second step reagent was Alexa Fluor® 555 (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X-100 and alcohol perm protocols.