

DESCRIPTION

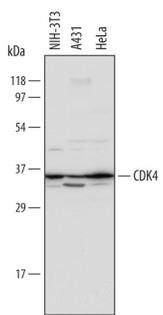
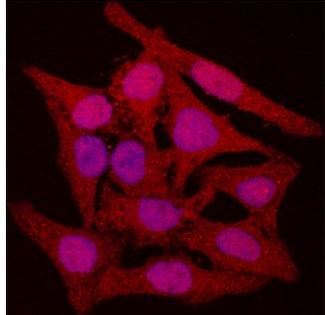
Species Reactivity	Human/Mouse
Specificity	Detects human and mouse CDK4 in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human CDK4 Met1-Glu303 Accession # P11802
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied 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	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Human and Mouse CDK4 by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma cell line, HeLa human cervical epithelial carcinoma cell line, and NIH-3T3 mouse embryonic fibroblast cell line. PVDF Membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse CDK4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5254) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for CDK4 at approximately 34 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>CDK4 in HeLa Human Cell Line. CDK4 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human/Mouse CDK4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5254) at 10 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 and nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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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. <ul style="list-style-type: none"> • 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

CDK4 (cyclin-dependent kinase 4) is a 34 kDa member of the CMGC Ser/Thr protein kinase family of enzymes. Within the cell, pRb/retinoblastoma protein is normally unphosphorylated and suppresses cell growth at the G1 stage of the cell cycle. When mitosis is required, CDK4 is phosphorylated, forms a complex with cyclin D1, 2 or 3, and phosphorylates pRb/retinoblastoma protein. This inactivates pRb and cells initiate DNA synthesis. Human CDK4 is 303 amino acids (aa) in length. It contains one protein kinase domain (aa 6-295) and an activating phosphorylation site at Thr172. There are three potential variants; one that shows an alternate start site at Met75, a second that shows a two aa substitution for aa 89-211, and a third that shows a 23 aa substitution for aa 89-303. Full-length human and mouse CDK4 are 95% aa identical.