Anti-Cdk4 Antibody [5-C3-G3]

M1505-5

Product Type:	Mouse monoclonal IgG2c, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 34 kDa
Clone number:	5-C3-G3

Description: Cell cycle progression is controlled in part by a family of cyclin proteins and cyclin dependent kinases (Cdks). Cdk proteins work in concert with the cyclins to phosphorylate key substrates involved in each phase of cell cycle progression . Another family of proteins, Cdk inhibitors, also plays a role in regulating the cell cycle by binding to cyclin-Cdk complexes and modulating their activity. Several Cdk proteins have been identified, including Cdk2-Cdk8, PCTAIRE-1-PCTAIRE-3, PITALRE and PITSLRE. Cdk4, in complex with D-type cyclins, is thought to regulate cell growth during the G1 phase of the cell cycle. This association with a D-type cyclin upregulates Cdk4 activity, whereas binding to the Cdk inhibitor p16 downregulates Cdk4 activity. Activation of the Cdk4-cyclin complexes requires phosphorylation on a single threonyl residue of Cdk4, catalyzed by a Cdk-activating protein (CAK).

Immunogen: Recombinant protein within human Cdk4 aa 80-303/303.

Positive control: A549 cell lysate, 293T cell lysate, HeLa cell lysate, MCF7 cell lysate, K-562 cell lysate, Mouse lung tissue lysate, NIH/3T3 cell lysate, C2C12 cell lysate, C6 cell lysate, PC-12 cell lysate, Rat lung tissue lysate, HeLa, human breast cancer tissue, human stomach cancer tissue, human thyroid cancer tissue, mouse colon tissue, mouse lung tissue, rat colon tissue, rat lung tissue.

Subcellular location: Cytoplasm, Nucleus, Nucleus membrane.

Database links: SwissProt: P11802 Human | P30285 Mouse | P35426 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:200-1:1,000
IF-Cell	1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

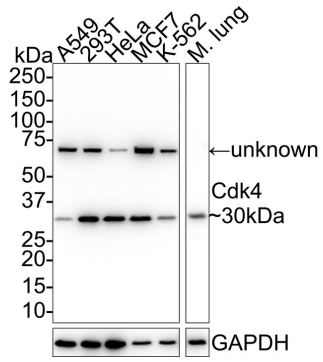

Fig1: Western blot analysis of Cdk4 on different lysates with Mouse anti-Cdk4 antibody (M1505-5) at 1/1,000 dilution.

Lane 1: A549 cell lysate (20 µg/Lane)
Lane 2: 293T cell lysate (20 µg/Lane)
Lane 3: HeLa cell lysate (20 µg/Lane)
Lane 4: MCF7 cell lysate (20 µg/Lane)
Lane 5: K-562 cell lysate (20 µg/Lane)
Lane 6: Mouse lung tissue lysate (40 µg/Lane)

Predicted band size: 34 kDa
Observed band size: 30 kDa

Exposure time: 17 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (M1505-5) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

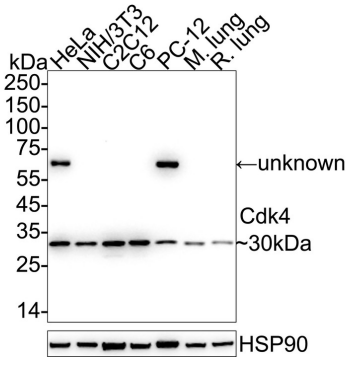

Fig2: Western blot analysis of Cdk4 on different lysates with Mouse anti-Cdk4 antibody (M1505-5) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: NIH/3T3 cell lysate (20 µg/Lane)
Lane 3: C2C12 cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)
Lane 5: PC-12 cell lysate (20 µg/Lane)
Lane 6: Mouse lung tissue lysate (20 µg/Lane)
Lane 7: Rat lung tissue lysate (20 µg/Lane)

Predicted band size: 34 kDa
Observed band size: 30 kDa

Exposure time: 20 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (M1505-5) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

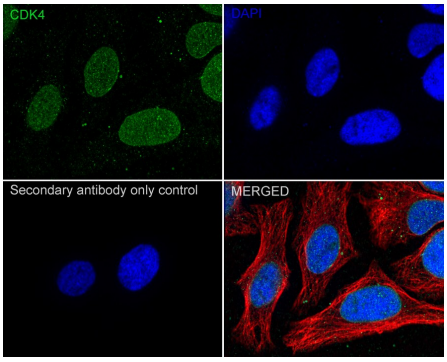

Fig3: Immunocytochemistry analysis of HeLa cells labeling Cdk4 with Mouse anti-Cdk4 antibody (M1505-5) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-Cdk4 antibody (M1505-5) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

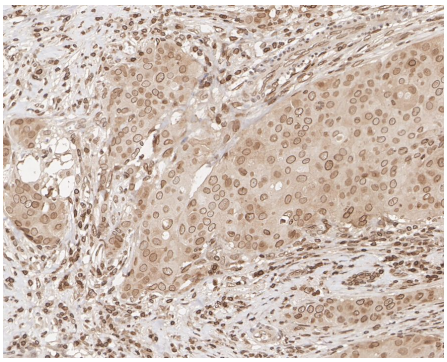

Fig4: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Mouse anti-Cdk4 antibody (M1505-5) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1505-5) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

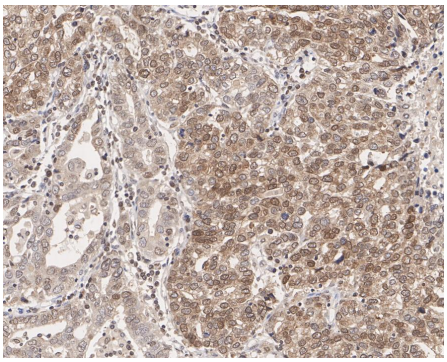

Fig5: Immunohistochemical analysis of paraffin-embedded human stomach cancer tissue with Mouse anti-Cdk4 antibody (M1505-5) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1505-5) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

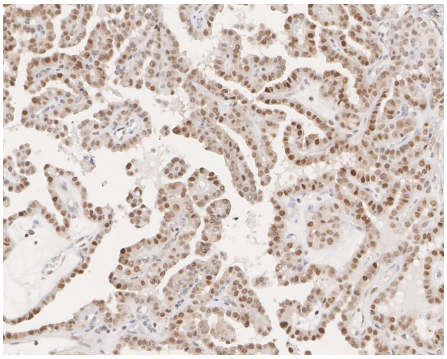

Fig6: Immunohistochemical analysis of paraffin-embedded human thyroid cancer tissue with Mouse anti-Cdk4 antibody (M1505-5) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1505-5) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

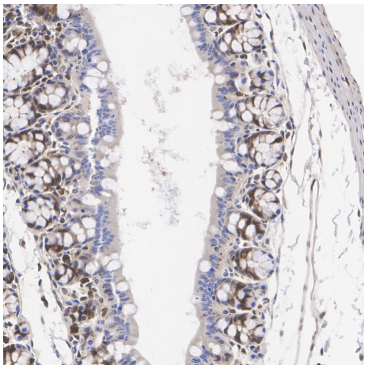

Fig7: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Mouse anti-Cdk4 antibody (M1505-5) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1505-5) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

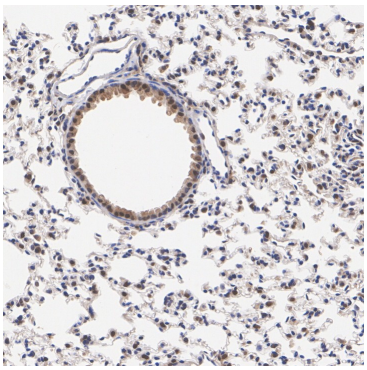

Fig8: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Mouse anti-Cdk4 antibody (M1505-5) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1505-5) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

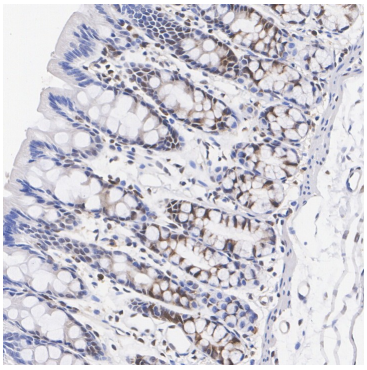

Fig9: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Mouse anti-Cdk4 antibody (M1505-5) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1505-5) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

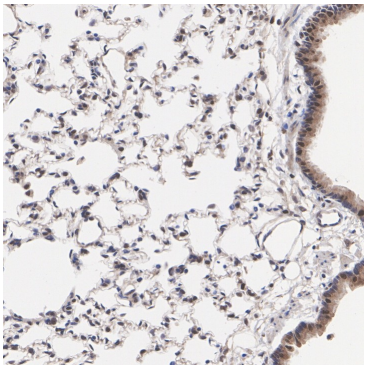

Fig10: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Mouse anti-Cdk4 antibody (M1505-5) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (M1505-5) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Wang Z et al. Migratory localization of cyclin D2-Cdk4 complex suggests a spatial regulation of the G1-S transition. Cell Struct Funct 33:171-183 (2008).
2. Bockstaele L et al. Differential regulation of cyclin-dependent kinase 4 (CDK4) and CDK6, evidence that CDK4 might not be activated by CDK7, and design of a CDK6 activating mutation. Mol Cell Biol 29:4188-4200 (2009).