Anti-CDKN2A/p16INK4a Antibody

ER1803-53

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 17 kDa

Description: This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene.

Immunogen: Synthetic peptide within C-terminal human CDKN2A/p16INK4a.

Positive control: HeLa cell lysate, HEK-293 cell lysate, Saos-2 cell lysate, 293T, SiHa, human colon cancer tissue, human stomach cancer tissue.

Subcellular location: Nucleus. Cytoplasm.

Database links: SwissProt: P42771 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Images

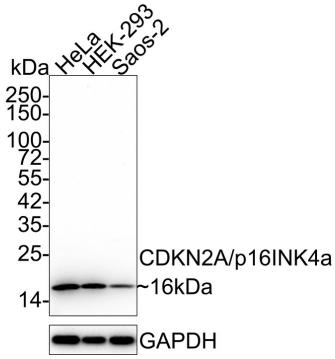

Fig1: Western blot analysis of CDKN2A/p16INK4a on different lysates with Rabbit anti-CDKN2A/p16INK4a antibody (ER1803-53) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: Saos-2 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 17 kDa
Observed band size: 16 kDa

Exposure time: 1 minute;

15% 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 (ER1803-53) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

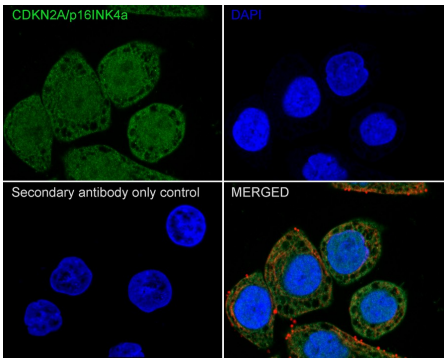

Fig2: Immunocytochemistry analysis of HeLa cells labeling CDKN2A/p16INK4a with Rabbit anti-CDKN2A/p16INK4a antibody (ER1803-53) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Rabbit anti-CDKN2A/p16INK4a antibody (ER1803-53) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

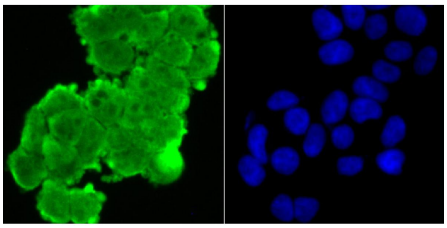

Fig3: ICC staining CDKN2A/p16INK4a in 293T cells (green). The nuclear counter stain is DAPI (blue). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.

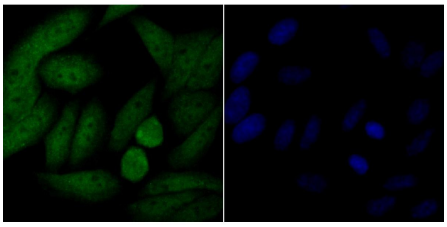

Fig4: ICC staining CDKN2A/p16INK4a in SiHa cells (green). The nuclear counter stain is DAPI (blue). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.

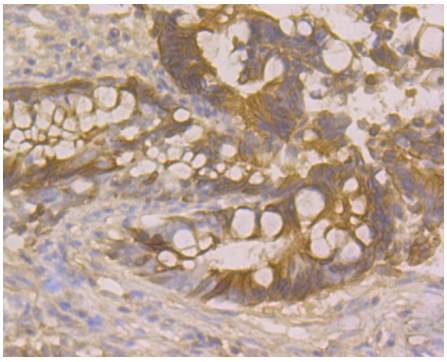

Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue using anti-CDKN2A/p16INK4a antibody. Counter stained with hematoxylin. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) for 20 mins.

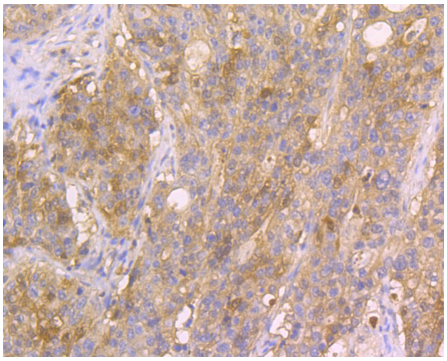

Fig6: Immunohistochemical analysis of paraffin-embedded human stomach cancer tissue using anti-CDKN2A/p16INK4a antibody. Counter stained with hematoxylin. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) for 20 mins.

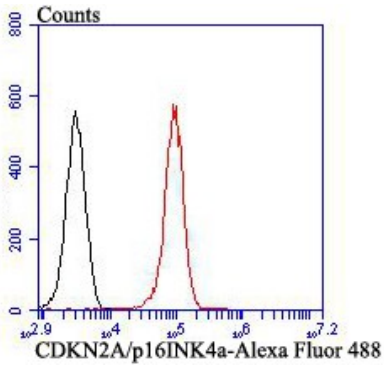

Fig7: Flow cytometric analysis of 293T cells with CDKN2A/p16INK4a antibody at 1/100 dilution (red) compared with an unlabelled control (cells without incubation with primary antibody; black). Alexa Fluor 488-conjugated goat anti-rabbit IgG was used as the secondary antibody.

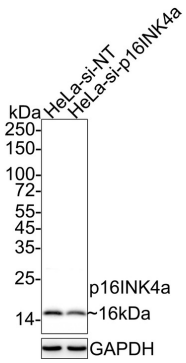

Fig8: Western blot analysis of CDKN2A/p16INK4a on different lysates with Rabbit anti-CDKN2A/p16INK4a antibody (ER1803-53) at 1/1,000 dilution.

Lane 1: HeLa-si NT cell lysate
Lane 2: HeLa-si p16INK4a cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 16 kDa
Observed band size: 16 kDa

Exposure time: 9 seconds;

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 (ER1803-53) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Okamoto A et al. Mutations and altered expression of p16INK4 in human cancer. Proc Natl Acad Sci USA 91:11045-11049 (1994).
2. Bockstaele L et al. Regulated activating Thr172 phosphorylation of cyclin-dependent kinase 4(CDK4): its relationship with cyclins and CDK 'inhibitors'. Mol Cell Biol 26:5070-5085 (2006).