Anti-p16INK4a Antibody [SU0702]

ET1608-62

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 16 kDa
Clone number:	SU0702

Description: p16 (also known as p16INK4a, cyclin-dependent kinase inhibitor 2A, CDKN2A, multiple tumor suppressor 1 and numerous other synonyms), is a protein that slows cell division by slowing the progression of the cell cycle from the G1 phase to the S phase, thereby acting as a tumor suppressor. It is encoded by the CDKN2A gene. A deletion (the omission of a part of the DNA sequence during replication) in this gene can result in insufficient or non-functional p16, accelerating the cell cycle and resulting in many types of cancer. p16 can be used as a biomarker to improve the histological diagnostic accuracy of grade 3 cervical intraepithelial neoplasia (CIN). p16 is also implicated in the prevention of melanoma, oropharyngeal squamous cell carcinoma, cervical cancer, vulvar cancer and esophageal cancer.

Immunogen: Synthetic peptide within Human p16INK4a aa 107-156 / 156.

Positive control: 293 cell lysate, SiHa cell lysate, HepG2, Hela, PC-3M, human colon carcinoma tissue, human cervical carcinoma tissue, human ovary carcinoma tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P42771 Human

Recommended Dilutions:

WB	1:500-1:1,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200
FC	1:50-1:100
IP	Use at an assay dependent concentration.

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

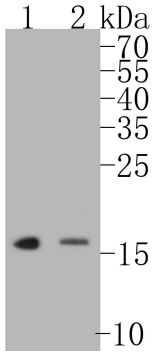

Fig1: Western blot analysis of p16INK4a on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1608-62, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:40,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: 293 cell lysate
Lane 2: SiHa cell lysate

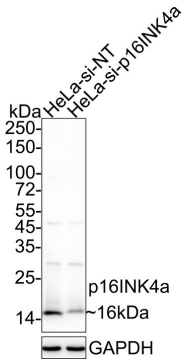

Fig2: Western blot analysis of p16INK4a on different lysates with Rabbit anti-p16INK4a antibody (ET1608-62) at 1/1,000 dilution.

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: 20 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 (ET1608-62) 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.

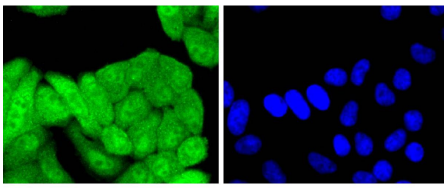

Fig3: ICC staining of p16INK4a in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1608-62, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

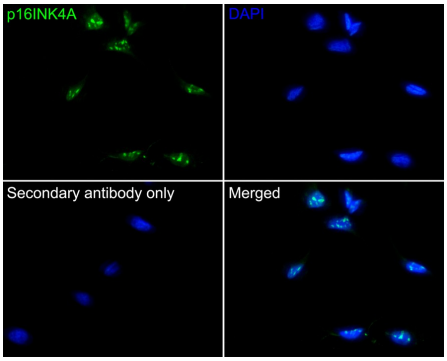

Fig4: Immunocytochemistry analysis of Hela cells labeling p16INK4a with Rabbit anti-p16INK4a antibody (ET1608-62) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-p16INK4a antibody (ET1608-62) at 1/50 dilution in 2% negative goat serum 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.

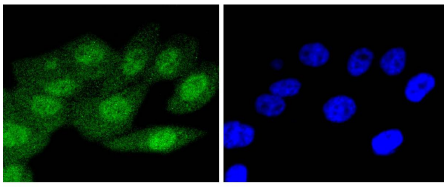

Fig5: ICC staining of p16INK4a in PC-3M cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1608-62, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

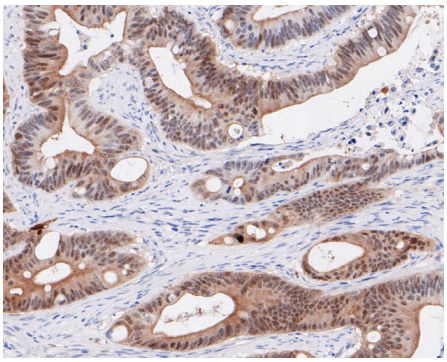

Fig6: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-p16INK4a antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-62, 1/50) for 30 minutes 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.

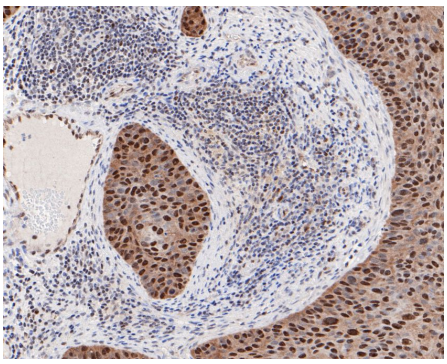

Fig7: Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue using anti-p16INK4a antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-62, 1/100) for 30 minutes 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.

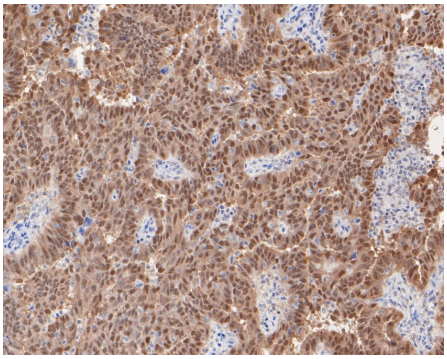

Fig8: Immunohistochemical analysis of paraffin-embedded human ovary carcinoma tissue using anti-p16INK4a antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-62, 1/100) for 30 minutes 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.

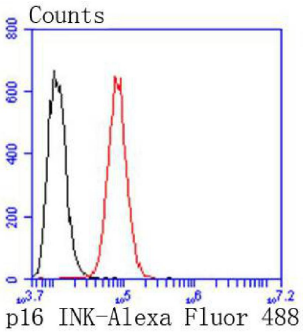

Fig9: Flow cytometric analysis of p16INK4a was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1608-62, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Background References

1. Idriss MH et al. Orthokeratotic Bowen disease: a histopathologic, immunohistochemical and molecular study. J Cutan Pathol 43:24-31 (2016).
2. Liang L et al. Assessment of the Utility of PAX8 Immunohistochemical Stain in Diagnosing Endocervical Glandular Lesions. Arch Pathol Lab Med 140:148-52 (2016).