# Anti-p16 Antibody [PD01-16]

## HA601131

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| **Product Type:** | Recombinant Mouse monoclonal IgG1, primary antibodies |
| **Species reactivity:** | Human |
| **Applications:** | IHC-P, WB, IF-Cell |
| **Molecular Wt:** | Predicted band size: 16 kDa |
| **Clone number:** | PD01-16 |

**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.

**Positive control:** Human tonsil tissue, human cervix poorly differentiated adenocarcinoma tissue, human cervix highly differentiated squamous cell carcinoma tissue, human ovary Advanced serous carcinoma tissue, HeLa cell lysate, HepG2 cell lysate, HEK-293 cell lysate, NIH:OVCAR-3 cell lysate, HeLa.

**Subcellular location:** Cytoplasm, Nucleus.

**Database links:** SwissProt: P42771 Human

**Recommended Dilutions:**

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| **IHC-P** | 1:200-1:500 |
| **WB** | 1:1,000 |
| **IF-Cell** | 1:100 |

**Storage Buffer:** PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

**Purity:** Protein A affinity purified.

## Images

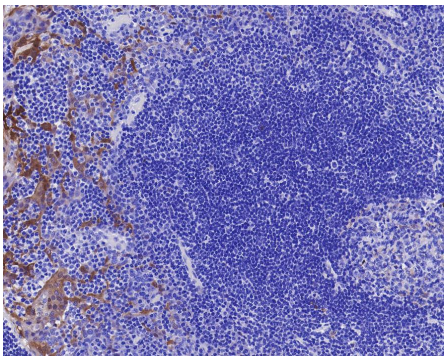

**Fig1:** Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Mouse anti-p16 antibody (HA601131) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA601131) at 1/500 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.

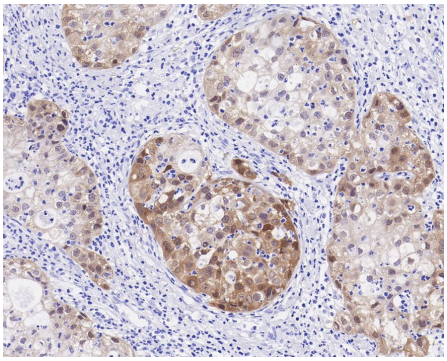

**Fig2:** Immunohistochemical analysis of paraffin-embedded human cervix
poorly differentiated adenocarcinoma tissue with Mouse anti-p16 antibody (HA601131) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA601131) at 1/500 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.

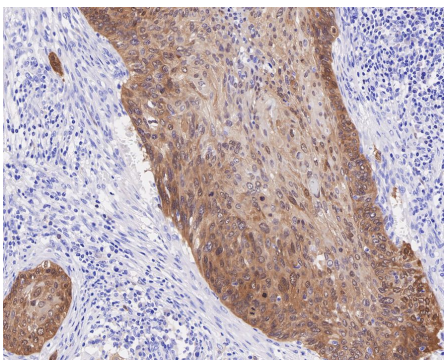

**Fig3:** Immunohistochemical analysis of paraffin-embedded human cervix
highly differentiated squamous cell carcinoma tissue with Mouse anti-p16 antibody (HA601131) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA601131) 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.

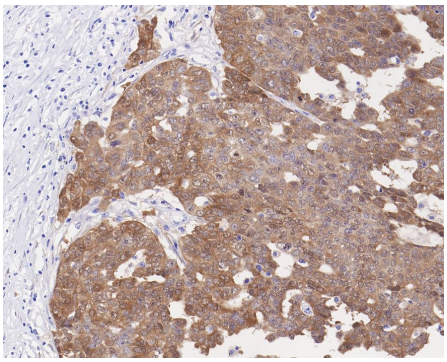

**Fig4:** Immunohistochemical analysis of paraffin-embedded human ovary
Advanced serous carcinoma tissue with Mouse anti-p16 antibody (HA601131) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (HA601131) at 1/500 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.

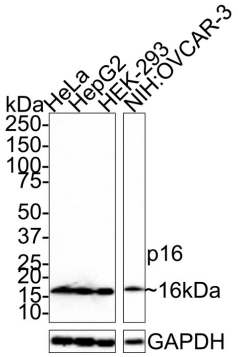


**Fig5:** Western blot analysis of p16 on different lysates with Mouse anti-p16 antibody (HA601131) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HepG2 cell lysate
Lane 3: HEK-293 cell lysate
Lane 4: NIH:OVCAR-3 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 2 minutes;

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 (HA601131) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

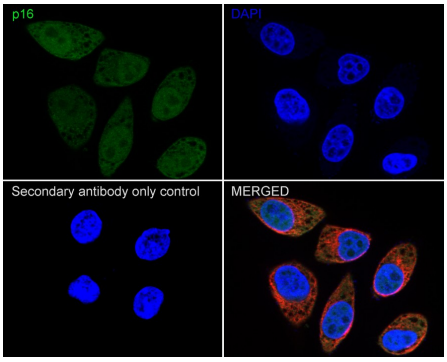


**Fig6:** Immunocytochemistry analysis of HeLa cells labeling p16 with Mouse anti-p16 antibody (HA601131) at 1/100 dilution.

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 Mouse anti-p16 antibody (HA601131) 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.

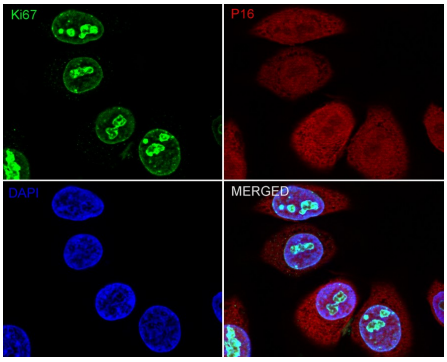


**Fig7:** Immunocytochemistry analysis of HeLa cells labeling p16 with Mouse anti-p16 antibody (HA601131) at 1/100 dilution.

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 Mouse anti-p16 antibody (HA601131) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) 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.

Ki67 (HA721115, green) was stained at 1/250 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution.



## Background References

1. Okamoto A. et. al. Mutations and altered expression of p16INK4 in human cancer. Proc. Natl. Acad. Sci. U.S.A. 91:11045-11049(1994).