

**Mouse anti-human p16<sup>INK4A</sup>**  
**monoclonal antibody (clone MM116)**  
Catalog Number: R16016M116

## General information

<b>IgG type</b>	Mouse IgG
<b>Clonality</b>	Monoclonal
<b>Applications</b>	IHC
<b>Specificity</b>	Human p16
<b>Formulation</b>	Tris Buffer, pH 7.3 - 7.7, with 1% BSA and 0.09% Sodium Azide
<b>Purity</b>	> 95% determined by SDS-PAGE
<b>Storage</b>	2-8 ° C (Do not freeze)

## Background

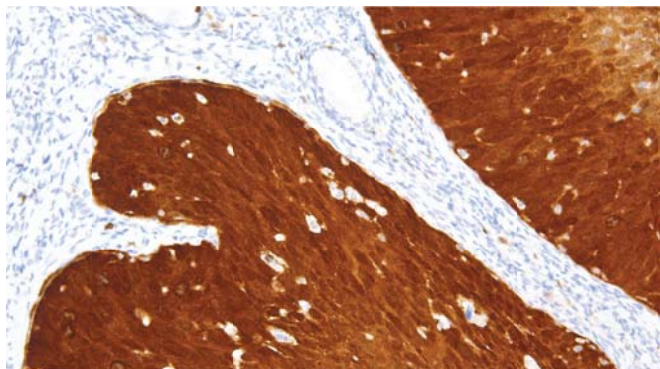
The p16 (p16<sup>INK4A</sup>) protein is a cyclin-dependent kinase (CDK) inhibitor that plays an important regulatory role in the cell cycle. By controlling the transition between the G1 and S phases through regulation of retinoblastoma protein, p16 decelerates cellular differentiation and therefore acts as a tumor suppressor, making it the key marker in several human cancers including head and neck cancer, perianal lesions, melanomas, gliomas, lymphomas, and some types of leukemia. p16 is also clinically indicated in carcinomas of the esophagus, pancreas, lung, biliary tract, liver, colon, and urinary bladder.

## Preparation

The concentrated antibody requires dilution in the optimized buffer, to the recommended working dilution range. The recommended working dilution range is 1:100 - 1:400.

## For Research or *in vitro* diagnostic (IVD) Use

## Application



p16<sup>INK4A</sup> [Cat. No: R16016M116] on Cervical Carcinoma

## Storage

This antibody is shipped at 4 °C. Store at 2-8°C. Do not freeze. When stored correctly, the antibody is stable until the date indicated on the label. To ensure proper stability and delivery of the antibody after each run, replace the cap and immediately place the bottle in a refrigerator in an upright position.

## Hazard/Biohazard

This antibody contains 0.09% sodium azide as preservative. Please handle and dispose the product properly. No known biohazard is associated with this product.

## Specimen Collection and Preparation for Analysis

Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4µm), and placed on a glass slide that is positively charged. The prepared slide may then be baked for a minimum of 30 minutes in a 53-65°C oven (do not exceed 24 hours).

*Note: Performance evaluation has been shown on human tissues only. Variable results may occur due to extended fixation time or special processes of specific tissue preparations.*

**Mouse anti-human p16<sup>INK4A</sup>  
monoclonal antibody (clone MM116)**  
Catalog Number: R16016M116



## Instructions For Use

*Recommended Staining Protocols for the p16<sup>INK4A</sup> [Cat. No: R16016M116] antibody:*

### Manual Use:

1. **Pretreatment:** Perform heat-induced epitope retrieval (HIER) at pH 9 for 10 to 30 minutes.
2. **Peroxide Block:** Block in peroxidase blocking solution for 5 minutes at room temperature. (Not required if using Alkaline Phosphatase System.)
3. **Primary Antibody:** Apply antibody directly (Predilute) or dilute antibody at 1:100-1:400 (Concentrate) before applying. Incubate antibody for 10 to 30 minutes at room temperature.
4. **Secondary Antibody:** Incubate for 20 to 30 minutes at room temperature.
5. **Substrate Development:** Incubate DAB or Fast Red for 5 to 10 minutes at room temperature.
6. **Counterstain:** Counterstain with hematoxylin for 0.5 to 5 minutes, depending on the hematoxylin used. Rinse with distilled water and blueing solution for 30 seconds.
7. Dehydrate and apply coverslip.

### Automated Staining System:

For all automated IHC staining systems, refer to the corresponding user manual for specific instructions.

## Quality Control Procedures and Interpretation of Results

The immunohistochemical staining process results in a colorimetric reaction at the site of the antigen, localized by the primary antibody. A qualified pathologist must interpret the patient results only once the positive and negative control tissues have been analyzed.

### Positive Control Tissue

A positive control tissue must be run with each staining procedure, and must be prepared and fixed identically to the test sections in order to provide control for all test variables, including fixation and tissue processing. The positive control tissue should be fresh autopsy, biopsy, or surgical specimens. For optimal quality control and to allow detection of lesser levels of reagent degradation, a tissue with weaker positive staining is advisable. Cervical carcinoma tissue can be used as positive control tissue for the p16<sup>INK4A</sup> [Cat. No: R16016M116] antibody. Where applicable, tissue that contains cells or tissue components that stain both positively and negatively may serve as both the positive and negative control tissue. Once stained, the positive control tissue should be analyzed to ensure appropriate positive staining is observed and all reagents are functioning properly. Positive reactivity requires the observation of an appropriate colorimetric reaction at the site of the antigen within the target cells. Counterstaining will result in a blue coloration, which may be pale to dark depending on the length of the incubation time

**Mouse anti-human p16<sup>INK4A</sup>  
monoclonal antibody (clone MM116)**  
**Catalog Number: R16016M116**



and potency of the hematoxylin. If positive staining as defined herein is not observed, the results obtained with the patient or tissue specimen must be treated as invalid. The positive control tissue should not be used as an aid in the diagnoses of patient samples, but rather solely as a measure of performance of the reagents and validity of obtained results.

### **Negative Control Tissue**

The same tissue used for the positive control tissue may be used as the negative control tissue. Most tissue sections offer internal negative control sites due to the diversity of cell types present, however this must be confirmed by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non-specific background staining. If specific staining is observed, the negative control tissue must be deemed invalid and the results obtained with the patient or tissue specimen must also be treated as such.

### **Patient Tissue**

Patient specimens should be analyzed only once the positive and negative control tissues have been deemed as valid. Negative staining indicates that the antigen was not detected; the use of a panel of antibodies may allow for recognition of false negative results, as negative staining in any one test does not confirm the absence of the antigen in question. A tissue section stained with hematoxylin and eosin should be used to analyze the morphology of the patient tissue sample, as verified by a qualified pathologist.

### **References**

1. Sano T, et al. *Am J Pathol.* 1998; 153:1741-8.
2. Agoff SN, et al. *Mod Pathol.* 2003; 16:665-73.
3. Negri G, et al. *Am J Surg Pathol.* 2003; 27:187-93.
4. Klaes R, et al. *Int J Cancer.* 2001; 92:276-84.
5. Klaes R, et al. *Am J Surg Pathol.* 2002; 26:1389-99.
6. Negri G, et al. *Virchows Arch.* 2004; 445:616-20.