IVD

ANTI- p16INK4a
(CLONE: 2D9A12)

CATALOG ID	DESCRIPTION
MM023-3D, 6D	3.0mL and 6.0mL RTU
MM023-AA, CC	0.2mL and 1.0mL Conc.

ALTERNATIVE NAME	p16Ink4A, CDKN2A, CDK 4 Inhibitor ,CDK 4 Inhibitor
CLONE	2D9A12
SPECIES	Mouse
ISOTYPE	IgG2b
TISSUE CONTROL	Human Brain carcinoma, Cervix and Ovarian carcinoma
EPITOPE/ IMMUNOGEN	p16INK4a
CELL LOCALIZATION	Cytoplasmic and Nuclear
SPECIES REACTIVITY	Mouse, Rat, Human
DILUTION RANGE	Assay dependent
DILUENT	Antibody Diluent Standard
Supplied as Buffer with protein carrier & preservative	

INTENDED USE

BioMarq p16INK4a antibody is used for *in vitro* diagnostic use only. This antibody is designed for the specific identification of p16INK4a protein in formalin-fixed paraffin-embedded tissue sections. The results using this product should be interpreted by a qualified pathologist in conjunction with the patient's relevant clinical history, other diagnostic tests and proper controls.

PRODUCT DESCRIPTION

The P16 (INK4) is a cyclin dependent kinase (Cdk) inhibitor protein. The cell cycle inhibitor proteins family includes P15 (INK4B) P16 (INK4A) P17 (INK4C) P18 (INK4D). P16 expression is commonly associated with cellular senescence. Progression of cell cycle is regulated by a family of Cdk proteins. P16 acts as a repressor for regulation of cell cycle. Its inhibiting activity is thought to be due to the formation of binary & ternary complex structures with Cdk proteins.

PRINCIPLE OF PROCEDURE

Immunohistochemistry (IHC) is a method for detecting antigens or haptens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. The antibody-antigen binding can be visualized in different methods. Enzymes, such as Horseradish Peroxidase (HRP) or Alkaline Phosphatase (AP), are commonly used to catalyze a color-producing reaction. IHC is widely used technique which makes it possible to visualize the distribution and localization of specific cellular components within cells and in proper tissue context. There are numerous IHC methods that can be used to localize antigens. The method selected should include consideration of parameters such as the specimen types and assay sensitivity.

IHC RECOMMENDED PROTOCOL

DeParaffinization & Hydration: DeParaffinization & Hydration is done using two grades of xylene & ethanol. Rinse in distilled water & follow next steps given

Pretreatment Solution: Perform heat Retrieval using BioMarq's Epitope Retrieval 1 (Catalog No PS001). (Refer to BioMarq's Epitope Retrieval 1 datasheet for specific instructions).

Peroxide Block: Incubate for 10 minutes with BioMarq EP Block (Catalog No BR001).

Protein Block (Optional): Incubate for 5-10 minutes at RT with BioMarq Protein Block (Catalog No BR002).

Primary Antibody: Incubate with Anti- p16INK4a antibody (Catalog No MM023) for 30-60 minutes at RT.

Probe: Incubate for 20 minutes at RT with a BioMarq Histochemistry probe (Catalog No HP001).

Secondary Antibody: Incubate for 20 minutes at RT with a BioMarq Polymer HRP antibody (Catalog No SA001).

Substrate/Chromogen: Incubate sections in DAB working solution for 5-7 minutes.

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Counterstain: Stain with BioMarq Mayer's Hematoxylin solution (Catalog No CS001) for 3-5min.

Mounting Solution: Mount the slides with BioMarq XY-Mount (Catalog No MS002) or using BioMarq T-Mount (Catalog No MS003).

TECHNICAL NOTE

This antibody staining has been standardized with BioMarq **IHC DETECTION KIT** (Catalog No DA001).

Ensure after each step slides are washed with BioMarq Immuno Wash Standard (Catalog No WB001) except peroxide Block step. Follow the instructions in the wash buffer data sheet for 1X solution preparation.

Follow the Antibody specific protocol recommendations provided in the data sheet. If atypical results occur, contact BioMarq Technical Support at 040-29702960.

STORAGE AND STABILITY

Store at 2-8°C. Do not freeze. Not to be used beyond the expiration date prescribed on label.

QUALITY CONTROL

For Quality Control purpose, each lot of this antibody is tested by immunohistochemistry using, formalin-fixed, paraffin-embedded **Cervix, Brain and Ovarian carcinoma** biopsy as control tissue. Users can also procure the Qualified Positive Control Slides available from BioMarq for their Quality Control purpose.

PRECAUTIONS

The material contains 0.05% Sodium azide as preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material.

Specimens should be handled carefully before and after the assay to avoid transmission of infection and disposed of with proper precautions

Microbial contamination of reagents may yield nonspecific staining.

For detailed safety information related to BioMarq Products, please refer to appropriate safety data sheets (SDS) available online at www.biomarq.net

LIMITATIONS

Factors which affect Immunohistochemical staining include the fixation process, Epitope-retrieval method, incubation times, tissue section thickness and detection kit used. Detection systems other than recommended by Biomarq when used results may vary due to the varied sensitivity of reagents and recommended incubation times. The recommendations and protocols mentioned in the datasheet are based on exclusive use of BioMarq products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist.

REFERENCES

1. Sawicka M et al, The Specificity and Patterns of Staining in Human Cells and Tissues of p16INK4a Antibodies Demonstrate Variant Antigen Binding, 2013 January.

2. Chiew-Loon Koo et al, Scoring mechanisms of p16INK4a immunohistochemistry based on either independent nucleic stain or mixed cytoplasmic with nucleic expression can significantly signal to distinguish between endocervical and endometrial adenocarcinomas in a tissue microarray study, J Transl Med, 2009 Apr.

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