MCM2 + TOP2A

Prediluted Antibody Cocktail
901-3181-092820

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Predilute	API 3181 AA, H	6.0, 25 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3181 T60	60 tests	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
MCM2 + TOP2A is a cocktail of mouse monoclonal antibodies that is intended for laboratory use in the qualitative identification of MCM2 and TOP2A proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
Reactive and regenerative squamous epithelium can show a spectrum of histologic alterations that mimic dysplastic and pre-neoplastic cytological and architectural changes. A monoclonal antibody cocktail targeted against mini-chromosome maintenance protein 2 (MCM2) and DNA topoisomerase IIA (TOP2A), when up regulated, serves as a marker of aberrant S-phase induction in proliferating cells (1). MCM2 functions during DNA replication by loading the pre-replication complex onto DNA and unwinding the DNA through helicase activity to permit DNA synthesis. MCM2 is essential for eukaryotic DNA replication and drives the formation of pre-replicative complexes, which is the key first step during G1 phase (2). Therefore, altered MCM2 expression may be a hallmark of cell-cycle deregulation, which could be the most essential mechanism in the development and progression of human cancers (2). This protein is overexpressed in cervical dysplasia as a result of HPV infection and subsequent uncontrolled activation of gene transcription and aberrant S-phase induction, which is mediated through the E2F transcription factor pathway. The overexpression of MCM2 provides the link between oncogenic HPV infection and the molecular event of cervical dysplasia (3,4). It has been shown that over-expression of MCM2 in cervical high grade dysplasia can be detected by immunohistochemistry (2). TOP2A is a nucleic enzyme that affects the topological structure of DNA by interacting with the double-helix DNA, thus playing an important role in DNA replication, transcription, recombination, condensation, and segregation (5,6). A monoclonal antibody cocktail directed against MCM2 and TOP2A is a novel immunostaining marker that has been tested in liquid-based cervical cytologic smears and cervical biopsy specimens as a potential diagnostic adjunct for the detection of high grade intraepithelial neoplasia and low grade intraepithelial neoplasia (7,8).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Reagent Provided:
MCM2 + TOP2A is provided as a prediluted antibody cocktail of anti-MCM2 and anti-TOP2A antibodies in buffer with carrier protein and preservative.

Antibody	anti-MCM2	anti-TOP2A
Clone	OT18A11	UMAB146
Source	Mouse Monoclonal	Mouse Monoclonal
Isotype	IgG2b	IgG1
Epitope/ Antigen	MCM2 (full-length)	TOP2A (aa 1100-1531)
Cellular Localization	Nuclear	Nuclear
Staining	Brown (DAB)	Brown (DAB)

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: Tonsil, normal cervix

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
Technical Notes:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Performance Characteristics:
Specificity and sensitivity on normal and diseased tissues are summarized in Tables 1 and 2, respectively.

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):
OPAI3181 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: MCM2+TOP2A
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Buffer Option): DS2-50
Antigen Retrieval (AR Option): AR1, high pH; 101°C
Block Option: Buffer
Reagent Name, Time, Temp.: MCM2+TOP2A, 30 min., 25°C

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Rev: 062117

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Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 211

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper
plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (9)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (10)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Walts AE, Bose S. p16, Ki-67, and BD ProEx™C immunostaining: a practical approach for diagnosis of cervical intraepithelial neoplasia. Hum Pathol. 2009;40:957.
2. Shi J, *et al*. Evaluation of p16INK4a, minichromosome maintenance protein 2, DNA topoisomerase IIA, ProEX C, and p16INK4a/ProEX C in cervical squamous intraepithelial lesions. Hum Pathol. 2007;38:1335.
3. Malinowski, DP. Molecular diagnostic assays for cervical neoplasia: emerging markers for the detection of high-grade cervical disease. Biotechniques. 2005;(Suppl 4):17.
4. Ishimi Y, *et al*. Enhanced expression of Mcm proteins in cancer cells derived from uterine cervix. Eur J Biochem. 2003;270:1089.
5. Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. Nat Rev Mol Cell Biol. 2002;3:430.
6. Gibbons D, *et al*. Comparison of topoisomerase II alpha and MIB-1 expression in uterine cervical squamous lesions. Mod Pathol. 1997;10:409.
7. Kelly D, *et al*. Detection of cervical high-grade squamous intraepithelial lesions from cytologic samples using a novel immunocytochemical assay (ProEx™C). Cancer. 2006;108:494.
8. Shroyer KR, *et al*. Validation of a novel immunocytochemical assay for topoisomerase II-a and minichromosome maintenance protein 2 expression in cervical cytology. Cancer. 2006;108:324.

References Cont'd

9. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
10. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Table 1: Specificity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebellum	0	1
Cerebral Cortex	0	1
Pituitary	0	1
Adrenal Gland	0	1
Thymus	1	1
Tonsil	1	1
Thyroid	0	1
Esophagus	0	1
Stomach	0	1
Small Intestine	1	1
Colon	1	1
Appendix	0	1
Pancreas	0	1
Spleen	0	1
Ovary	0	1
Cervix	13	13
Endomyometrium	1	1
Fallopian Tube	0	1
Placenta	1	1
Kidney	0	1
Bladder	0	1
Urethra	0	1
Breast	1	1
Prostate	0	1
Testis	1	1
Myocardium	0	1
Smooth Muscle	0	1
Skeletal Muscle	0	1
Lymph Node	1	1
Aorta	0	1
Bone Marrow	1	1
Lung	0	1
Skin	1	1
Liver	0	2

Note: Macrophages are stained in many tissues. Cervical basal layer stained.

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Table 2: Sensitivity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Lung Squamous Cell Carcinoma	2	2
Lung Adenocarcinoma	2	2
Breast Cancer	4	4
Colon Cancer	4	4
Prostate Cancer	4	4
Cervical Dysplasia (I, II, III)	9	9

Note: Tumor cells are stained focally and weakly to moderately with high heterogeneity.

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