IVD

HRP/DAB DETECTION KIT – ONE STEP
(IHC DETECTION KIT)

CATALOG ID	DESCRIPTION
DA001-60	60 Tests Kit

INTENDED USE

BioMarq IHC Detection Kit is intended for *in vitro* diagnostic identification of Rabbit primary IgG antibody and mouse primary IgM antibodies. This HRP-DAB IHC Detection Kit aids in localization of antigens in a broad range of histological specimens.

PRODUCT DESCRIPTION

BioMarq IHC detection kit is a pre-manufactured simplified kit for Immunohistochemistry. The kit obviates the need for each individual clinical laboratory to validate each reagent as pre-optimized working solutions and recommended working protocols are provided

BioMarq IHC detection system is highly sensitive and specific non-biotin, micro-polymer based one-step detection system which significantly reduces or shows no back ground on tissues containing high levels of endogenous biotin. This system is based on an HRP labeled polymer, which is conjugated with secondary antibodies.

PRINCIPLE OF PROCEDURE

Immunohistochemistry (IHC) technique is widely used for detection of antigens in histological specimens. The basic principle of IHC is the use of enzyme-linked antibodies to detect tissue antigens. The colorless substrate is converted by enzyme into a colored product that precipitates on the slide at the site of the antigen localization.

BioMarq IHC detection kits use DAB (3, 3'-diaminobenzidine) Chromogen as a substrate of Horseradish peroxidase (HRP) for visualization of antigenic structures in the tissues. This substrate produces a brown color product which is insoluble in alcohol. The high sensitivity and specificity of BioMarq Detection system is achieved by using nonspecific IgG's-absorbed secondary biotinylated antibodies and sensitive streptavidin conjugated to HRP. Kits work on both FFPE and frozen tissue sections.

KIT CONTENTS:

EP Block	**2 Vials X 6mL**
Protein Block	**2 Vials X 6mL**
Polymer HRP Antibody	**1 Vial X 6mL**
DAB Substrate Buffer	**1 Vial X 10mL**
DAB Chromogen	**1 Vial X 0.2mL**
Empty Mixing Vial	**1 Vial**
Mayer's Hematoxylin	**2 Vials X 6mL**

MATERIALS AND REAGENTS (NEEDED BUT NOT PROVIDED):

Microscope slides (Catalog No SL001-72 & SL002-72)

Oven

Xylene/ Xylene substitute

Reagent alcohol/Ethanol

Pressure cooker/ Microwave/ Steamer

Pretreatment Solutions (Epitope retrieval Solutions)

Coplin Jars/Staining Containers

Deionized or distilled water

Wash buffer

Primary antibody

Positive controls

Mounting Solutions

DAB WORKING SOLUTION PREPARATION

DAB WORKING SOLUTION: Add 20.0µl of liquid DAB chromogen (SC001) to 1.0mL of DAB Substarte buffer (SB001) in empty Mixing vial provided (MV001) and mix for few seconds.

DAB Working Solution is stable at Room Temperature for 8hours. For best result s use within 4 hours of preparation.

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RECOMMENDED PROTOCOL

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

PRETREATMENT SOLUTION: Unmasking of antigens/epitopes in paraffin sections can be accomplished by using pretreatment /Epitope retrieval solutions. Always refer to the primary antibody data sheet of BioMarq for recommended Pretreatment Solution.

STAINING PROCEDURE:

PEROXIDE BLOCK: Add BioMarq EP Block (~ 200µl) to the tissue sections and incubate for 10 minutes. Wash slides 2-3 times with Immunowash Standard Wash Solution.

PROTEIN BLOCK: Add BioMarq Protein Block (~ 200µl) to the tissue sections and incubate for 10 minutes.

PRIMARY ANTIBODY: Incubate the slides with BioMarq primary antibody as per the specifications mentioned in the antibody data sheet.

POLYMER-HRP ANTIBODY: Add BioMarq Polymer HRP Antibody (~ 100µl) to the tissue sections and incubate for 30 minutes. Wash slides 2-3 times with Immunowash Standard Wash buffer.

DAB SUBSTRATE SOLUTION: Add DAB Substrate solution (~ 100µl) to the tissue sections and incubate for 5-10 minutes. Wash slides 2-3 times with Immunowash Standard Wash buffer.

COUNTERSTAIN: Counterstain the tissue sections with BioMarq Mayer's Hematoxylin (~ 200µl) solution 5-10 minutes. Wash slides 2-3 times with Immunowash Standard Wash buffer.

MOUNTING: Dehydrate the slides with graded alcohol & Xylene/Xylene substitute. Mount the tissue slides using BioMarq XY or T-Mount for permanent storage.

STORAGE AND STABILITY

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

QUALITY CONTROL

BioMarq IHC detection kits show reproducible and consistent results when used with BioMarq Rabbit/Mouse antibodies (Both Monoclonal and Polyclonal) with high sensitivity and specificity. Also the kit ensures minimal to no background when protocols are strictly followed.

For Quality Control purpose, each lot of this Detection Kit is tested by Immunohistochemistry using BioMarq's Primary Antibodies and Reagents.

TECHNICAL NOTE

Do not interchange reagents of this kit with components from any other BioMarq or other vendor detection kits.

Any changes in the kit staining procedures (dilution, washing, incubation time or temperature) can alter the performance.

Bring the reagents to **Room Temperature** before adding to the sections.

Prepare working solutions shortly before use and discard the leftover.

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

EXPECTED RESULTS

BioMarq IHC detection kit results in intense, clear staining at the antigen sites in both the specimen and positive control. For any deviation from these expected results refer to the troubleshooting guide mentioned below.

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TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	SUGGESTION
SECTIONS DETACHED FROM SLIDES	Excessive washing	↓ wash steps / Time. Avoid excessive shaking of slides during washing
	Slides not suitable for IHC	Use positively charged slides or Silane coated slides
DETERIORATION OF TISSUE SECTIONS	Incomplete fixation	↑ fixation time. Use fresh fixative
WEAK OR NO STAINING (POOR SIGNAL)	Deparaffinization incomplete	↑ Deparaff. Time. Use fresh solvent
	Epitope retrieval incomplete	Use right Epitope retrieval
	Inappropriate fixative or incomplete fixation	Follow recommended fixation time & use right fixative
	Primary Ab conc &/or incubation time is too low	↑ Ab conc &/or incubation time
	Primary Ab not suitable for IHC or tissue type (FFPE/Frozen).	Use Ab recommended for IHC & tissue sections.
	Expired Kit reagents	Use new kit
	Presence of enzyme inhibitors	Use buffer free of sodium azide.
HIGH BACKGROUND (POOR SIGNAL: NOISE RATIO)	Inadequate washing	↑ washing steps or time
	Primary Ab conc &/or incubation time is too high	↓ Ab conc &/or incubation time
	Non-specific binding	↑ incubation time with serum blocking reagents
	Sections dried during staining	Work quickly to avoid drying of sections.
	High content of endogenous peroxidase in tissue	↑ conc and/or incubation time of EP Block.

PRECAUTIONS

DAB Buffer is harmful. If contact with skin, wash with plenty of water. In case of contact with eyes, rinse immediately with water and seek medical advice.

DAB chromogen is classified as a carcinogenic product. Wear gloves and protective clothing to avoid contact with skin.

Microbial contamination of reagents may yield nonspecific staining.

Dispose the reagents (DAB & other) as per applicable Federal and state regulations.

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 primary antibodies used. Primary Antibodies/Materials/Reagents 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. Carson, F.L. Histopathology: A self-Instructional Text. ASCP Press, Chicago, 1990.

2. Bullock, G.R., and Petrusz, eds (1990) Techniques in immunohistochemistry, Vol 2: Academic Press: New York.

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