

CITRTAE BUFFER (pH 6.0) (Epitope Retrieving Solution for IHC)

CATALOG ID	DESCRIPTION
PS003-KD	1000 mL Ready-to-use
PS003-CC	100 mL Concentrated (10X)

INTENDED USE

BioMarq Citrate Buffer (pH 6.0) is used for *in vitro* diagnostic use only. This buffered Retrieval Solution is intended for heat induced Antigen Retrieval of formalin-fixed paraffin-embedded tissues for IHC procedures.

PRODUCT DESCRIPTION

BioMarq Citrate buffer (pH 6.0) is used as a heat-induced antigen retriever on formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies.

BioMarq Citrate buffer (pH 6.0) is most widely preferred heat induced Antigen retrieval solution compatible with all antibodies (Rabbit/Mouse and Monoclonal/Polyclonal).

In immunohistochemistry (IHC), most commonly used fixatives such as formalin mask tissue antigens (cellular, membrane, and nuclear) by their intrinsic crosslinking. This masking of antigens results in poor or no staining in IHC. The use of BioMarq Citrate buffer, pH 6.0 on FFPE tissue sections improves accessibility of antibodies to tissue antigens.

PRINCIPLE OF PROCEDURE

Formaldehyde fixation usually generates methylene bridges which cross-link proteins and therefore mask the epitope of interest. Antigen retrieval methods break these methylene bridges and expose antigenic sites, allowing antibodies to bind. Two methods frequently used for antigen retrieval are heat induced epitope retrieval (HIER) and enzymatic retrieval. As enzymatic retrieval causes damage to the morphology of the section, heat-induced epitope retrieval is often preferred choice of retrieval.

HIER is performed using a pressure cooker, a microwave, or a steamer.

Antigen retrieval technique depends on multiple variables, including but not limited to, the target antigen, the antibody used, the type of tissue, and the method and duration of fixation. Biomarq offers various types of Epitope Retrieval Solutions to detect antigens in tissues with enhanced immunoreactivity.

PREPARATION OF WORKING SOLUTION

1. Mix 1 part concentrated buffer to 9 parts Deionized Water (1:10 dilution).

- OR -

Dilute contents of the 10X Citrate Buffer (100 ml) with 900 mL of Deionized Water.

MATERIALS AND REAGENTS (NEEDED BUT NOT PROVIDED):

Microscope slides
 Oven
 Xylene/ Xylene substitute
 Reagent alcohol/Ethanol
 Pressure cooker/ Microwave/ Steamer
 Deionized or distilled water
 Wash buffer
 Peroxide block
 Protein block
 Primary antibody
 Tissue controls
 Detection kits
 Haematoxylin
 Mounting Solutions

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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: Immerse the slides in BioMarq's Citrate Buffer (pH 6.0) (Catalog No PS003) solution placed in a slide container/ Coplin Jar.

Heat slides in solution for 15-20 minutes at 100°C. Alternatively the slides can be heated in solution under pressure for 10-20 minutes.

Remove and cool the slides at room temperature for 20 minutes.

Gently rinse slides with DI water

Antibody Staining: Proceed for further Antibody staining protocol as specified in Antibody datasheet.

STORAGE AND STABILITY

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

QUALITY CONTROL

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

TECHNICAL NOTE

Occasionally the buffer may develop yellowish tinge due to added preservative and will not affect performance of buffer.

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

PRECAUTIONS

Do not refrigerate Citrate Buffer; this will cause a precipitate to form.

Avoid skin and eye contact, inhalation, and ingestion.

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. Kanai K, Nunoya T, Shibuya K, Nakamura T, Tajima M. Variations in effectiveness of antigen retrieval pretreatments for diagnostic immunohistochemistry. Res Vet Sci.; 1998, 64(1):57-61.
2. Brown RW, Chirala R. Utility of microwave-citrate antigen retrieval in diagnostic immunohistochemistry. Mod Pathol; 1995, 8(5):515-20.