# I. INTENDED USE

For Research Use Only.

Antigen Retrieval Buffer is intended for the enhancement of chromogenic detection of targeted antigens that have been reacted with a user-supplied primary antibody and a detection system.

#### **II. INTRODUCTION**

Heat induced epitope restoration, or HIER, is an effective method of restoring antigenic epitopes that have been adversely affected by tissue processing and fixation procedures. In most formalin-fixed, paraffin-embedded tissue sections, HIER pretreatment is essential for optimal IHC results. The Antigen Retrieval Buffer, containing Tris buffered EDTA at pH 9.0-9.5, is specially formulated to be reliable, effective and universal for the restoration of antigenic epitopes on formalin-fixed, paraffin-embedded tissues that require HIER pretreatment.

#### **III. HANDLING, STORAGE AND SHELF LIFE**

Storage Conditions: All reagents are to be stored at 2-8°C.Void after expiration date as specified on kit/reagent label.

**Precautions:** 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. The user is advised to consult the MSDS for further information.

# IV. REAGENTS AND MATERIALS NEEDED BUT NOT SUPPLIED

Primary antibodies or Detection systems

Related products available from Leica Biosystems

1) PowerVision™ Poly-HRP and Poly-AP IHC Detection Systems: biotin-free

2) PowerVision+™ Poly-HRP and Poly-AP IHC Detection Systems: biotin-free, highly sensitive

3) Primary antibodies

# V. PROCEDURES

Tissue fixation can have adverse effects on antigenicity, leading to false-negative staining or high background. Antigen retrieval pretreatment is the most effective way to restoring antigenicity and in most cases, also reducing backgrounds. Always consult the primary antibody data sheet for pretreatment information.

The following is a suggested protocol for HIER. The combination of antigen retrieval, antibody incubation and detection should be determined by the user.

1) Dilute one part Antigen Retrieval Buffer with 9 parts of deionised water

2) Immerse slides in the diluted buffer

3) Apply heat from a hot plate until boiling and maintain gentle boiling for 20 minutes

4) Turn off heat and, while keep slides still immersed in the solution, let solutions cool off naturally for 40-60 minutes

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5) Rinse slides with wash buffer and proceed as suggested by primary antibody and detection data sheets.

# VI. LIMITATIONS

Antigen Retrieval Buffer helps restoring antigenic epitopes while reducing non-specific background in IHC procedures. Correct treatment of tissues prior to fixation and embedding is important for obtaining optimal results. Inconsistent results may be due to variation in fixation, embedding, antibody reactivity, as well as from inherent variations in tissue. Leica Biosystems warrants that the materials sold meet our performance specifications until the expiration. No other warranties or guarantees, expressed or implied, are provided, including warranties for merchantability or fitness for a particular purpose.

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