10X Citrate Buffer *pH 6.0*

Catalog number: 10000
Unit size: 100 mL

Component	Storage	Amount (Cat No. 10000)
10X Citrate Buffer *pH 6.0*	Refrigerated (2-8 °C)	100 mL

OVERVIEW

Citrate Buffer is prepared from citric acid and sodium citrate and functions as a weak acid buffer system for maintaining acidic pH conditions. Citric acid is a triprotic acid with three dissociation constants (pKa values of approximately 3.13, 4.76, and 6.40), enabling effective buffering within the pH range of about 3.0 to 6.2. This buffering system is widely used in biochemical and histological workflows where controlled acidic conditions are required. A common application of citrate buffer is in heat-induced epitope retrieval (HIER) procedures used for formalin-fixed paraffin-embedded (FFPE) tissue samples. During aldehyde fixation, protein crosslinks may form that alter the conformation of antigenic sites and reduce accessibility for antibodies or nucleic acid probes. Heating tissue sections in citrate buffer solutions, typically in the range of 0.01–0.1 M, is widely used to help reduce fixation-induced crosslinks and restore antigen accessibility, improving downstream detection in immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) assays.

AT A GLANCE

1. Deparaffinize FFPE tissue sections and hydrate with appropriate buffer.
2. Immerse slides in 1X buffer and heat at 100 °C for 10–20 minutes or in a pressure cooker for 3–5 minutes.
3. Cool at room temperature for ~20 minutes and proceed with the IHC staining protocol.

PREPARATION OF WORKING SOLUTION

Dilute 10X buffer as needed (e.g., 10 mL of 10X Citrate buffer + 90 mL of distilled water), and mix well.

Note: 1X citrate buffer can be stored at 2-8°C.

SAMPLE EXPERIMENTAL PROTOCOL

1. Deparaffinize FFPE tissue sections according to the standard laboratory deparaffinization protocol.
2. Rehydrate the tissue sections with PBS or universal immuno buffer.
3. Prepare a 1X working solution of the buffer. Immerse the tissue slides in the 1X solution and perform heat-induced antigen retrieval using one of the following methods:
   - Heat at 100 °C for 10–20 minutes, or
   - Heat in a pressure cooker for 3–5 minutes.
4. Allow the slides to cool at room temperature for approximately 20 minutes.
5. After cooling, the tissue sections are ready to proceed with the subsequent immunohistochemistry (IHC) staining protocol.

Note: The buffer may exhibit a slight yellowish coloration due to the presence of preservatives. This does not affect the performance of the buffer.

Note: Tissue sections derived from brain tissue or tissues with high lipid content may detach from slides or show loss of tissue morphology during heating. In such cases, the use of positively charged, silanized, or polylysine-coated slides is recommended.

EXAMPLE DATA ANALYSIS AND FIGURES

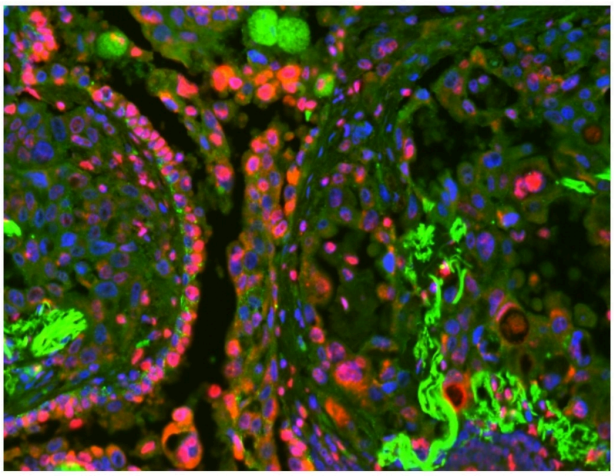

Figure 1. Multiplex immunohistochemistry (IHC) with sequential staining and antigen retrieval. Tissue was first stained with HDAC-2 (3F3) mouse primary antibody, followed by HRP-labeled Goat Anti-Mouse IgG (Cat. #16728) and detection using iFluor® 647 Styramide (Cat. #45045). Antibodies were then stripped using 10X Citrate Buffer (Cat. #10000) before a second staining cycle with EpCAM (VU1D9) mouse antibody, HRP-labeled Goat Anti-Mouse IgG (Cat. #16728), and iFluor® 488 Styramide (Cat. #45020). Nuclei were counterstained with DAPI (Cat. #17510), enabling multiplex visualization of HDAC-2 and EPCAM in the same tissue section.

DISCLAIMER

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