

## Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20)

### Instructions For Use

These instructions apply to the WellMed Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20).

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#### 1: Intended Use

For In Vitro Diagnostic and Research Use. WellMed Citrate buffer is intended for use in immunohistochemistry for heat-induced epitope retrieval.

#### 2: Summary and explanation

WellMed Citrate buffer, is designed for use during heat-induced epitope retrieval (HIER) on formalin-fixed paraffin-embedded tissue sections prior to antibody application. In immunohistochemistry (IHC), protein cross-links are formed during formalin or other aldehyde fixation. These cross-links mask the antigenic sites in tissue specimens and results in weak or false negative staining in immunohistochemical detection of proteins. The use of this citrate buffer in combination with heat ensures that the antigenicity of proteins modified, thus enhancing staining intensity of antibodies. For the recommended pre-treatment technique, please refer to the individual antibody's instructions for use. This buffer is a 10x concentrate solution and must be diluted 1:10 with deionized water before using. The final ready-to-use solution should have a pH of  $6.0 \pm 0.3$ . The buffer can be used for a maximum of three runs within five days after dilution.

### 3: Availability

Catalog Number	Contents	Volume
HIER1000CC	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20) , Clear	1000 ml
HIER1000CR	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20), Red color	1000 ml
HIER500CC	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20) , Clear	500 ml
HIER500CR	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20), Red color	500 ml
HIER250CC	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20) , Clear	250 ml
HIER250CR	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20), Red color	250 ml
HIER100CC	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20), Clear	100 ml
HIER100CR	Citrate buffer, Heat-Induced Epitope Retrieval (10x, Tween20), Red color	100 ml

### 4: Recommended usage

Before use dilute 1:10 with deionized water. The final ready-to-use solution should have a pH of 6.0 ± 0.3.

Protocol:

1. Deparaffinize and rehydrate the tissue slides.
2. Fill the PT Module tank with 150 ml of citrate buffer and 1,350 ml of deionized water, to obtain 1,500 ml of ready-to-use solution (1:10 dilution).
3. Pre-heat the PT Module until temperature reaches 65°C and place the formalin-fixed slides in slide racks into the PT Module tank.
4. Heat the citrate buffer and slides to 98°C and incubate for 20 minutes.
5. Cool slides in the PT Module to 65°C.
6. Remove slides and cool to room temperature for at least 5 minutes in a PBS or TBS based buffer.
7. Continue with staining according to IHC protocol.

**Note:** Alternative heating sources, such as a microwave or a pressure cooker, can be used to replace the PT Module tank. The optimal incubation time for these heating sources should be determined by authorised personnel / researchers.

## 5: Warnings and precautions

Refer to SDS.

## 6: Storage

Store at room temperature. Do not use after the expiration date.

## 7: Microbiological state

Product(s) not sterile.

## 8: Supplied as

10x concentrate solution, in clear and red.

## 9: Troubleshooting

Please contact WellMed by phone or by email.

## 10: Reference

[1] Morgan JM, Navabi H, Schmid KW, Jasani B (1994) Possible role of tissue-bound calcium ions in citrate-mediated high-temperature antigen retrieval. *J Pathol.* 174(4):301-7.

[2] Hoffman, E. A., Frey, B. L., Smith, L. M., & Auble, D. T. (2015). Formaldehyde Crosslinking: A Tool for the Study of Chromatin Complexes. *Journal of Biological Chemistry*, 290(44), 26404–26411.

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