Avda. Conocimiento, 100
P.T. Ciencias de la Salud
18016 Granada
Fax: 958.27.14.34
Tlf.: 958.27.14.49
www.masterdiagnostica.com

EDTA buffer (10x concentrated) pH 8.0

PRESENTATION:

Concentrated buffer solution (10 x), with a final pH of 8.0. Presentations for this product are:

Reference	Concentrated solution (mL)	Final diluted solution (mL)
MAD-004072R/D	1000	10000

INTENDED USE: IVD Product for in vitro diagnostic use

SPECIFICITY, INTERFERENCE AND LIMITATIONS:

EDTA buffer is used in methods for heat induced antigen retrieval (HIER). When used on material fixed in buffered formalin and embedded in paraffin, certain antibodies require antigen unmasking for retrieving the immunoreactivity that had been masked by the fixing process.

APPLICATIONS:

The HIER has improved immunohistochemical staining in conventional surgical pathology as it improves the antigen unmasking and the staining intensity. The use of these techniques has its best advantages in case of nuclear antigens that presents a marked increase in signal intensity.

PRODUCT COMPOSITION:

EDTA pH8.0.

RECOMMENDATIONS FOR USE:

Before use: The EDTA solution is 10x concentrated and therefore it msut be diluted in a 1:10 range with distilled or deionized water (one part EDTA buffer and 9 parts water).

1. - Cut and mount sections on either charged or silane coated slides.

2A. - In case of using an automated PT Module for simultaneous dewaxing, rehydrating and antigen (HIER) retrieval follow the instructions of use enclosed in the product data sheet. Basically fill the tanks with 1.5 litres of diluted, ready-to-use buffer solution, place the slides without dewaxing into the racks and then immerse them into the buffer solution. Program the PT Module for a cycle of antigen retrieval of 20min at 95ºC and then cool to room temperature. Then follow with the step 4

2B. - In case of not using the PT Module for HIER continue with dewaxing (using xylene or substitute) and rehydration of the tissue sections through decreasing series of ethanol. Then follow with the step 3 and 4.

3. - Unmasking: various alternative methods could be used to carry out the antigen retrieval process using the diluted EDTA buffer:

2.1 Unmasking in microwave: Place the sections in the diluted solution of EDTA (Coplin jar or other container with a volume as large as the laboratory considers) and bring the microwave oven at 700 watts (watt) for 10-15 minutes. Cool to room temperature.

2.2 Unmasking in pressure cooker: Fill the pressure cooker with 1-2 litres of dilute EDTA buffer. While hydrating the slides, heat the solution avoiding boiling. Then immerse the sections in the preheated buffer and close the pressure cooker. Wait until the pressure rises to the second notch and run from here 1-2 minutes (depending on the antigen unmasking). Remove and cool the pot (you can speed up the process placing the pot under running water). Open when there is no pressure and cool the slides inside of the pot for 20-30 minutes.

4. - Remove the sections and place them into the usual washing buffer (recommended TBS / TBS-Tween pH 7.5 – reference MAD-004077R)

5. - Proceed with the immunostaining protocol as usual.

SAFETY RECOMMENDATIONS

This product is intended for laboratory professional use only. The product is NOT intended to be used as a drug or for domestic purposes. The current version of the Safety Data Sheet for this product can be downloaded by searching the reference number at www.vitro.bio or can be requested at regulatory.md@vitro.bio.

STORAGE:

Store until the expiry date of the product at room temperature, away from any sources of intense heat or cold.

BIBLIOGRAPHY:

1. Norton A.J., et al; Brief, high temperature heat denaturation (pressure-cooking): a simple and effective method of antigen retrieval for routinely processed tissues. *J Pathol* 173 (4): 371-391(1994).
2. Clive R. Taylor, M.D., Ph.D., Shan-Rong Shi, M.D., and Richard J. Cote, M.D. Appl Inmunohistochem 4 (3): 144-166, 1996
1. Suthipintawong C., et al; Immunostaining of cell preparations: a comparative evaluation of common fixatives and protocols. *Diagn Cytopathol* 15: 167-174 (1996)