Pretreatment Module™ Deparaffinization and Heat-Induced Epitope Retrieval Solution

Please note this datasheet has been changed effective Dec 3, 2008

INTENDED USE

For In Vitro Diagnostic Use

AVAILABILITY:	Catalog #	Volume
PT Module™ Buffer 1 (100X Citrate Buffer, pH 6.0)	TA-250-PM1X	250 ml (100X)

SPECIFICITY: N/A
ENZYME: N/A
CHROMOGEN/SUBSTRATE: N/A

DESCRIPTION

This product is designed to deparaffinize and perform Heat Induced Antigen Retrieval (HIER) on formalin-fixed paraffin-embedded tissue sections mounted on glass microscope slides.

~~Deparaffinizat~~ion is accomplished by melting the paraffin at the high temperature used in HIER (over 90°C) along with ~~emulsifica~~tion of the paraffin by a detergent (1).

Formaldehyde fixation may impair the immunoreactivity of many antigens and epitopes. The negative effect of formaldehyde fixation can be reversed successfully with enzymatic digestion for some markers while not for others. Non-enzymatic epitope unmasking techniques using heated buffer solutions at various pH levels has been reported to improve the reactivity of many antibodies in formalin-fixed tissues (2,3).

The product is designed to be used in the Lab Vision Pretreatment Module™. Determination of use conditions in any other instrument is the responsibility of the user. The Lab Vision Pretreatment Module™ is designed to be used with slide racks used in the Lab Vision 360, 480 and 720 Autostainers, Microm 710i Autostainer, BioCare Nemesis 7200 Autostainer and the DAKOCytomation Autostainer and AutostainerPlus. Use of any other slide holding technology is the responsibility of the user.

WARNINGS & PRECAUTIONS

Refer to MSDS.

STORAGE & SHELF LIFE

Store at room temperature. Each component is stable for 18 months. This buffer contains no preservative. Store product at 2-8°C for storage longer than 3 months. Discard if solution becomes cloudy or shows a large amount of precipitate.

MICROBIOLOGICAL STATE

Product(s) not sterile.

MATERIALS REQUIRED BUT NOT PROVIDED

Pretreatment Module™ (Lab Vision Corp. Cat# PT-Module).

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SPECIMEN & REAGENT PREPARATION

Refer to Procedure.

PROCEDURE

Supplied As:
Buffer solution of various pH levels. This is a 100X stock solution and should be diluted 100-fold with distilled water before use. Depending on pH of water, the pH of the solution may require pH adjustment. The diluted solution should be ±0.2 pH units of the stated pH.
Use the proper pH solution for the antibody being detected. The proper solution to use for a given antibody must be determined by the user.

NOTE: For all steps concerning the PreTreatment Module™ (PTM) please refer to PTM manual.

1. Place five-micron thick tissue sections on glass slides treated to enhance tissue adherence. Dry slides at 37°C overnight or 60°C for one hour. Sections should be thoroughly dry before proceeding.
2. Fill each of the Pretreatment Module (PTM) tanks with 1.5L of the appropriate PTM buffer.
3. Program PTM to preheat to 60°C and heat to 98°C for 20 minutes (or use protocol deemed optimal in your laboratory).
4. Start preheat cycle (ideally the PTM is preheated before slides are placed in the solution. This saves time).
5. Mount the slides into the Autostainer racks.
6. Place slide racks into the PTM.
7. Press RUN on the PTM to start the heating cycle. The PTM will heat to the desired temperature and then cool down to 60°C. A beep will sound when the program is finished (about one hour total).
8. Take racks out of PTM and place in Autostainer.
9. Wash slides well with either warm (60-80°C) Autostainer buffer or distilled water.
10. Proceed with desired Autostainer program.
11. Dispose of solution according to local disposal regulations. See MSDS. Do not reuse solution.

Suggested Working Dilution:
1:100 with distilled water

Suggested Test Size:
1.5L of solution is used in each PTM tank. Use 15ml buffer per 1485ml distilled water to fill one PT Module tank.

REFERENCES

1) Epitope Retrieval Technique: *A simple modification that reduces staining time*. Yorukoglu K, et al. Appl Immunohistochem 5(1):71, 1997.
2) Shi, S-R, Key ME, Kalra KL. *Antigen retrieval in formalin-fixed paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections*. J Histochem Cytochem 1991;39:741-8.
3) Shi,et.al. *Antigen Retrieval Immunohistochemistry: Practice and Development*. J Histotech; Vol 20;No 2:145-158.

TROUBLESHOOTING

Sparse paraffin on slide: dots or crystals of paraffin	Rinse Slides well with hot (80°C) Autostainer buffer or distilled water.
Extensive paraffin dots/crystals	1) Solution is saturated with paraffin. Use fresh PTM buffer on each run. Do not reuse solution. 2) Paraffin has high polymer content and polymer is not fully removed. May not be suitable for aqueous deparaffinization techniques.
Poor deparaffinization: White patches of no staining	Areas of thick paraffin may not fully deparaffinize. This is caused primarily by pooling of paraffin when it melts while slide drying. Be sure paraffin is not pooled over tissue after drying/melting in oven

Please contact Thermo Fisher Scientific Technical Support by phone (1-510-991-2800 or 1-800-828-1628) or by email (lab.reagents@thermofisher.com).

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