PA-Retrieval Solution(pH9.0)

REF WF18P0001

INTENDED USE

PA-Retrieval Solution(pH9.0) is a ready to use epitope retrieval solution for the heat-induced epitope retrieval (HIER) of formalin-fixed, paraffin-embedded tissue sections prior to immunohistochemical staining on the Fully Automated Pathology Staining System (Model No.: PA-3600).
For *in vitro* diagnostic use only. For professional use only.

SUMMARY

The use of a HIER pretreatment on formalin-fixed, paraffin-embedded tissue on the PA-3600 automated system restores epitopes that have been modified by formalin fixation, allowing accessibility of the primary antibody to the epitope.

PRINCIPLE

Fixation of tissue by formalin results in the formation of covalent bonds between the aldehyde and amino groups present in the tissue. The formation of these bonds denatures protein and can result in the loss of accessibility of epitopes or target nucleic acids. In addition, the formaldehyde can form methylene bridges cross linking tissue proteins thus reducing the penetration of the tissue to large molecules such as antibodies.
PA-Retrieval Solution(pH9.0) is a Tris-based buffer with a slightly basic pH, which, at elevated temperatures is capable of hydrolyzing the covalent bonds formed by formalin in tissue. Removing these bonds allows renaturation of protein molecules and increases antibody or probe accessibility. Often these changes result in significant gains in antibody or probe binding and improved signal to noise ratios.

PRECAUTION

1. This kit is for *in vitro* diagnostic use only.
2. Do not reuse, expired products may not be used.
3. The kit is to be used by professionals.
4. Insufficient amount of reagents in the experiment may lead to incorrect results.
5. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
6. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
7. Ensure that the waste container is empty prior to starting a run on the instrument. If this precaution is not taken, the waste container may overflow and the user risks a slip and fall.
8. Unused solution should be disposed of in accordance with all local, regional, national and international regulations.
9. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

MATERIALS

Materials Provided

For WF18P0001（100 Tests）

Contents	Main components	Quantities
Retrieval Solution pH9.0	Retrieval Solution pH9.0 contains a Tris-EDTA based buffer.	15 mL/bottle * 3
Instruction for Use	Instruction for Use	1

Materials Required but Not Provided

1. PA-Sample Release Reagent
2. PA-Immunochromogenic Reagent
3. PA-Bluing Reagent
4. PA-Enhancer(Linker)
5. PA-Wash Buffer
6. Microscope slides
7. Positive and negative tissue to use as process controls
8. Distilled or de-ionized water
9. Ethanol absolute
10. Xylene or xylene substitutes
11. Permanent mounting medium
12. Cover glass
13. General purpose laboratory equipment
14. Bright field microscope (4-40x objective magnification)
15. 7ml reagent vial (Tagged with RFID)

Equipment Needed

Fully Automated Pathology Staining System (Model No.: PA-3600).

STORAGE AND STABILITY

1. Store at 2~8°C, valid for 18 months
2. Keep away from sunlight, moisture and heat.
3. Freezing and thawing prohibited.
4. Use within 3 months after opening.
5. Tighten the cap and return to 2~8°C immediately after use.
6. Do not use after expiration date printed on the vial label.

SPECIMEN COLLECTION AND PREPARATION

The specimens may be formalin-fixed, paraffin-embedded tissue sections. Fixation time is dependent on fixative and tissue type/thickness. For example, tissue blocks with a thickness of 3~5 mm should be fixed in neutral-buffered formalin for 18～24 hours.

The optimal thickness of paraffin-embedded sections is approximately 3~5 μm. After sectioning, tissues should be mounted on Microscope Slides and then placed in a 65 ± 2°C calibrated oven for 1 hour.
The sections should be mounted on the slides as flat and wrinkle-free as possible. Wrinkles will have a negative impact on the staining results.
NOTE: The position of the specimens on the microscope slides must suitable for the PA3600 instrument. Please refer to the User Guide for definition of usable staining area of the microscope slide for the specimen.

TEST PROCEDURE

Used in combination with the Fully Automated Pathology Staining System (Model No.: PA-3600), the process of dewaxing to counterstaining is completed by the instrument.
1. Place the prepared slides in a 65 ± 2°C calibrated oven for 1 hour.
2. Following the operating instructions of the instrument software.
3. Setting up the protocol using the instrument software and printing labels.
4. Loading the labeled slides into the instrument.
5. Placing reagents into the reagent rack and confirming that the reagent type is correct and that the amount of reagents is sufficient to complete the experiment.
6. Start the operation for automatic staining.
7. After staining is completed, remove the sections and rinse with distilled water.
8. Then dehydrate and transparent the slides, finally mount the slides with permanent mounting medium and cover slipped.

For complete information and operating procedure, please refer to PA-3600 Operation Manual.

RESULT INTERPRETATION

Expected results are quantitative only when testing the sensitivity and specificity of each specific antigen or target nucleic acid sequence. As a standalone reagent, this product cannot be tested for specificity or sensitivity.
Multiple Wondfo primary antibodies have been developed with PA-Retrieval Solution(pH9.0) in IHC, ICC and ISH applications. As part of the testing for those assays, the following performance characteristics were demonstrated for PA-Retrieval Solution(pH9.0):

1. Within-run, between-day and night, and between-instrument precision on the PA-3600 instruments.
2. Sensitivity and specificity of staining across a range of normal and neoplastic tissue types and assay-specific target tissues.

All studies met their acceptance criteria.

QUALITY CONTROL

Positive control should be set for each batch of experiments to

monitor the operation process and the quality of reagents; a positive control can be fished on Refer to Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline-Second Edition (I/LA28-A2) CLSI. 2011.

Positive and negative control tissues (lab-supplied) should be run for each staining procedure. These quality controls are intended to ensure the validity of the staining procedure, including reagents, tissue processing and instrument performance. It is recommended that control tissues be stained on the same slide as the patient tissue.

Positive Control
The positive control should be a tissue with positive biomarker expression. External Positive control materials should be fresh specimens fixed, processed, and embedded as soon as possible in the same manner as the patient sample(s). One positive external tissue control for each set of test conditions should be included in each staining run.
If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Control
The negative control should be a tissue or tissue element with no biomarker expression. Use a negative tissue control fixed, processed, and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the IHC primary antibody for demonstration of the target antigen, and to provide an indication of specific background staining (false positive staining).
If specific staining (false positive staining) occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Nonspecific Negative Reagent Control
Use a nonspecific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site.
The incubation period for the negative reagent control should correspond to that of the primary antibody.

LIMITATIONS OF PROCEDURE

1. Immunohistochemistry is a multi-step process, each step may influence the result, these include, but are not limited to fixation, antigen retrieval method, incubation time, tissue section thickness, detection kit used and interpretation of the staining results.
2. The recommended protocols are based on exclusive use of Wondfo products.
3. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist.
4. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

PERFORMANCE CHARACTERISTICS

Positive conformance
The positive control was taken, and the immunohistochemical test was carried out according to the instructions of the manufacturer. The results met the requirements that the positive tissue/cell staining result should be positive, the positioning of the positive staining should be accurate, and there should be no background staining or non-specific staining.

Negative conformance
Take a negative control according to the manufacturer's instructions for immunohistochemical tests, the results met the negative tissue/-cell staining results of negative, no background staining or non-specific staining requirements.

Blank control conformance
Take a blank control according to the manufacturer's instructions for immunohistochemical tests, and antibody diluent was used instead of primary antibody working liquid as a blank control. Immunohistochemical tests were conducted according to the instructions of the manufacturer. The results met the requirements of negative, no background and non-specific staining of the infected tissues/cells.

Intra batch precision
Three tissue slices from the same tissue source containing the target antigen were taken and the same batch of products were used for immunohistochemical detection. The results met the requirements of no obvious difference in staining intensity and localization of tissue slices from the same tissue source.

Inter batch precision
Three tissue slices from the same tissue source containing the target antigen were taken and 3 different batches of products were used for immunohistochemical detection at the same time. The results met the requirements that there is no obvious difference in the intensity and location of staining of tissue slices from the same tissue source with different batches of reagents.

BIBLIOGRAPHY

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[2] Wu Bingquan, Liu Yanfang. Immunohistochemical Pathological diagnosis (2nd edition) [M]. Science in Beijing Technology Press, 2013.
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[4] JD Bancroft and A Stevens. Theory and Practice of Histological Techniques. 4th Edition. Churchill Livingstone, New York. 1996.
[5] Clinical and laboratory Standards Institute (CLSI). Protection of Laboratory workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

INDEX OF SYMBOL

	Consult instructions for use		Manufacturer		Date of manufacture
IVD	*In vitro* diagnostic medical device		Use-by date	REF	Catalogue number
	Temperature limit	LOT	Batch Code		Contains sufficient for <n> tests
	Keep away from Sunlight	Vol.	Abbreviation for volume	CE	CE marking
	Importer	UDI	Unique device identifier	EC REP	Authorized representative in the European Community/ European Union

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