PA-Immunochromogenic Reagent (Dual staining)-RUO

REF WF39P0001

INTENDED USE

PA-Immunochromogenic Reagent (Dual staining) is a simple and robust one-step polymer detection system for visualization of two antigens simultaneously in brown and red, respectively. The double stain detection system contains alkaline phosphatase (AP) and horseradish peroxidase (HRP); it provides excellent sensitivity and saves time when used with simultaneous double staining. The system is biotin-free; background staining caused by endogenous biotin is completely eliminated. It is intended for staining sections of formalin-fixed, paraffin-embedded tissue on the Fully Automated Pathology Staining System (Model No.: PA-3600).
For research use only.

SUMMARY

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells. Prior to staining, formalin-fixed, paraffin-embedded tissue sections should be subjected to deparaf-finization followed by heat-induced epitope retrieval (HIER) using the target retrieval method specified in the Instructions for Use for the primary antibody. Endogenous peroxidase should be blocked with Block Buffer included in the PA-Sample Release Reagent kit. Primary antibodies are not provided with the kit. A primary antibody cocktail of choice may be used in conjunction with ready-to-use HRP/AP polymer, composed of anti-mouse/HRP + anti-rabbit/AP.
The substrate system in the kit consists of two types: fast red and DAB. The substrate system produces crisp red and brown end products in depending on the species of the primary antibody, respectively. Hematoxylin counterstaining provides a clear blue, nuclear staining. Using PA-Immunochromogenic Reagent (Dual staining) in combination with the Fully Automated Pathology Staining System reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

PRINCIPLE

The PA-Immunochromogenic Reagent (Dual staining) Kit detects specific mouse and rabbit primary antibodies bound to an antigen in formalin-fixed, paraffin-embedded (FFPE) tissue sections. The mouse primary antibody is located by a specific HRP-labeled secondary antibody. The complex is then visualized with DAB, which produces a brown precipitate. The rabbit primary antibody is located by a specific HRP-labeled secondary antibody. The complex is then visualized with fast red, which produces a red precipitate.

PRECAUTION

1. This kit is for research 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. ProClin 300 solution is used as a preservative in this solution. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
6. Avoid contact of reagents with eyes, skin, and mucous membranes. Use disposable gloves and wear suitable protective clothing when handling suspected carcinogens or toxic materials.
7. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
8. 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.
9. PA-Immunochromogenic Reagent (Dual staining) contains material of animal origin. As with any product derived from biological sources, proper handling procedures should be used according to local requirements.
10. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
11. Unused solution should be disposed of in accordance with all local, regional, national and international regulations.
12. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent author- ity of the Member State in which the user and/or the patient is established.

MATERIALS

Materials Provided

For WF39P0001 (100 Tests)

Contents	Main components	Quantities
HRP/AP Polymer	HRP/AP Polymer contains Anti-rabbit Poly-HRP-IgG (approximately 30 μg/mL) and Anti-rabbit Poly-AP-IgG (approximately 30 μg/mL) in a buffer containing protein with 0.05% ProClin 300.	15 mL/bottle * 1
Red substrate	Red substrate contains ≤1% (w/v) naphthol in a proprietary stabilizer buffer solution.	15 mL/bottle * 1
Red chromogen	Red chromogen contains ≤1% Fast red in a proprietary stabilizer solution.	15 mL/bottle * 1
DAB substrate	DAB substrate contains ≤0.1% (v/v) hydrogen.	15 mL/bottle * 1
DAB chromogen	peroxide in a proprietary stabilizer buffer solution.	15 mL/bottle * 1
Hematoxylin	Hematoxylin contains ≤0.1% (w/v) Hematoxylin in a glycol and acetic acid stabilizing solution.	15 mL/bottle * 1
Instruction for Use	Instruction for Use	1

Materials Required but Not Provided

1. PA-Sample Release Reagent
2. PA-Retrieval Solution(pH9.0)
3. PA-Retrieval Solution(pH6.0)
4. PA-Bluing Reagent
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. Aqueous 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 in distilled water.

8. Finally mount the slides with aqueous mounting medium and cover slipped.

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

RESULT INTERPRETATION

The fast red-containing Substrate Working Solution gives a red color at the site of the target antigen recognized by the rabbit primary antibody. The DAB-containing Substrate Working Solution gives a brown color at the site of the target antigen recognized by the mouse primary antibody. If nonspecific staining is present, this will be recognized as a rather diffuse, red or brown staining on the slides treated with the negative control reagent. Nuclei will be stained blue by the hematoxylin counterstain.

QUALITY CONTROL

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. Immunohisto-chemical 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 require-ments 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.

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.