# AExVisual® Enhanced Poly-HRP Goat Anti-Mouse,Rabbit IgG

Instructions for Use | Cat. No.: ARX1021

## 1. Intended Use

This product is intended for use in immunohistochemical staining. It binds to the primary antibody associated with the target antigen and enables chromogenic labeling of the target site.

## 2. Principle of the Test

This product is used for staining paraffin-embedded tissue sections. Paraffin-embedded tissues are subjected to deparaffinization, heat-induced antigen retrieval, and staining to support observation of tissue morphology and the distribution of specific proteins. The reagent uses polymer technology in which enzyme molecules are linked to a multi-branched polymer structure, forming a dense cascade-like complex. This increases enzyme-labeling density, provides broader compatibility with tissue fixation methods and fixation times, and reduces variation caused by differences in tissue collection and processing. The procedure is designed to be convenient, rapid, sensitive, and reproducible.

## 3. Main Components

| Component | Composition |
|---|---|
| Enhancer | Goat anti-mouse IgG, goat anti-rabbit IgG, buffer, and preservative. |
| Polymer | Polymerized enzyme-labeled rabbit anti-goat IgG, buffer, and preservative. |

Purified water, absolute ethanol, and the primary antibody reagent are not provided. Dispensing of samples and reagents, heating, draining, water filling, and related actions may be completed automatically by the instrument.

## 4. Storage Conditions and Shelf Life

- Store at 2-8°C. Do not freeze.
- Shelf life is 18 months when stored under the specified conditions.
- Manufacturing date: see product label.
- During use, remove the reagent only when needed and return it to refrigerated storage immediately after use. After opening, the reagent is valid for 6 months.
- The product may be transported in a sealed foam container with ice packs for no more than 7 days. Product performance is not affected if the unpacking temperature does not exceed 30°C.

## 5. Applicable Instrument

Fully automated immunohistochemistry staining instrument.

## 6. Specimen Requirements

- This product is applicable to paraffin-section specimens processed in accordance with pathological technical requirements and prepared within 3 years.
- Specimens with a diameter of >=2 cm should be cut at intervals of 1 cm after surgery and fixed in neutral formaldehyde solution within 30 minutes. A fixation time of 8-24 hours is recommended to support reliable subsequent immunohistochemical testing.
- The recommended tissue section thickness is 3-5 μm. Sections should be mounted on anti-detachment glass slides. Sections may be stored for 1 year at 2-8°C.
- Applicable specimen types include routine paraffin sections and frozen sections.

## 7. Manual Test Procedure

Required instruments and equipment: pipette, incubator, immunohistochemistry pen, timer, incubation box, staining rack, coverslip, optical microscope, and wash bottle.

Reagent preparation: the chromogenic working solution shall be prepared immediately before use.

Procedure: after antigen retrieval, paraffin sections shall be stained as follows:

1. Circle the tissue area with an immunohistochemistry pen and promptly add wash solution to prevent the section from drying.
2. Add 3% H2O2 to the tissue within the marked area, using enough reagent to cover the tissue. Incubate for 10 minutes, then wash with wash solution.
3. Add the primary antibody working solution and incubate in a humidified chamber for 30 minutes. The incubation time may be adjusted based on experimental results. Wash 3 times with wash solution.
4. Remove as much wash solution as possible. Wipe away wash solution outside the marked area with tissue paper to prevent Enhancer overflow. Add 1-2 drops of Enhancer, using enough reagent to cover the tissue. React for 10 minutes, then wash 3 times with wash solution.
5. Remove as much wash solution as possible. Wipe away wash solution outside the marked area with tissue paper to prevent Polymer overflow. Add 1-2 drops of Polymer, using enough reagent to cover the tissue. React for 10 minutes, then wash at least 6 times with wash solution. If time permits, soak for 30 seconds to 1 minute during washing.
6. Prepare DAB working solution by adding 76 µL of Solution B (1x) to a centrifuge tube, then adding 4 µL of Solution A (20x) and mixing. Use approximately 80 µL per slide. Apply the DAB working solution with a pipette to cover the tissue for 1-5 minutes, then rinse 3 times with purified water.
7. Add 1-2 drops of hematoxylin for 10-30 seconds. Wash with wash solution, then rinse with purified water. If required, treat with bluing solution for 3-5 seconds, then rinse with tap water.
8. Dehydrate slides through graded alcohols (75%, 95%, and 100%). Dry the slide surface with an air blower, add mounting medium, and apply a coverslip.
9. Examine microscopically.

## 8. Interpretation of Results

| Result | Interpretation |
|---|---|
| Positive | Brown-yellow staining is observed for the target antigen in the target cells of the tested tissue (DAB chromogenic reaction). |
| Negative | No brown-yellow staining is observed for the target antigen in the target cells of the tested tissue (DAB chromogenic reaction). |

## 9. Limitations of the Test Method

1. Immunohistochemistry is a multi-step detection process, including selection of appropriate reagents; tissue collection, fixation, processing, and slide preparation; and interpretation of staining results. Users must receive professional training.
2. Tissue staining depends on pre-staining processing. Inappropriate fixation, freezing and thawing, washing, drying, heating, sectioning, or contamination by other tissues or fluids may cause artificial false-negative results. Inconsistent results may be caused by differences in fixation and embedding methods or by intrinsic tissue heterogeneity.
3. Excessive or insufficient counterstaining may interfere with accurate interpretation of results.
4. Clinical interpretation of results shall be made in conjunction with the patient's clinical presentation, morphology, histopathological criteria, and appropriate positive and negative controls.
5. A negative result in immunohistochemical testing indicates that the antigen was not detected; it does not indicate that the antigen is absent from the tested cells or tissue.

## 10. Performance Characteristics

1 Conformity: for qualified tissue sections, including positive and negative tissue controls, positive staining shall show accurate localization with no background staining. Blank controls and negative controls shall be negative.

2 Within-batch repeatability: staining intensity and localization shall show no obvious difference among tissue sections from the same tissue source.

3 Other performance characteristics comply with the product technical requirements specified by the enterprise.

## 11. Precautions

1 This product is for scientific research use only and shall not be used for other purposes.

2 Use appropriate protective measures to avoid contact with skin and eyes.

3 DAB has sensitizing potential. Wear gloves during operation, avoid skin contact as much as possible, and rinse thoroughly after use. The chromogenic reagent contains DAB; reagent bottles shall be kept tightly closed to avoid leakage. In the event of leakage, handle with gloves and avoid direct skin contact.

4 Incomplete deparaffinization may affect staining performance. It is recommended that deparaffinization of immunohistochemistry sections be performed separately from routine HE deparaffinization.

5 Positive and negative controls shall be included during the test procedure to prevent possible false-positive or false-negative results.

6 Excess PBS buffer during reagent addition may dilute the reagent and weaken staining intensity. Remove excess buffer before adding reagents.

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