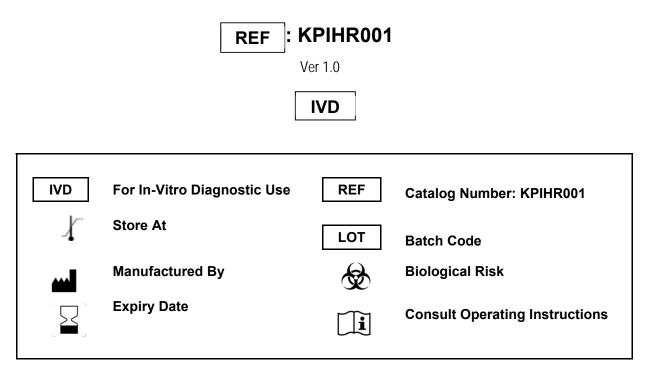
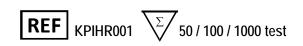
# **Polymer-HRP IHC Detection System**



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## Introduction

Antigen detection by immunohistochemistry (IHC) is a two-step process wherein the primary antibody binds to specific epitopes of the antigen of interest and that binding is detected by a chromogen. KRISHGEN Polymer-HRP Detection System uses a non-streptavidin-biotin proprietary micropolymer-complex technology to minimize background staining wherein an antibody enhancer/amplifier and a polymer-HRP reagent are bound to the primary antibody and visualized by diaminobenzidine (DAB).

## Principle

The Polymer-HRP Detection System is a biotin-free, polymeric horseradish peroxidase (HRP)- secondary antibody conjugate system for the detection of mouse and rabbit primary antibody on formalin-fixed, paraffinembedded (FFPE) tissues in an immunohistochemistry (IHC) procedure. The innovative technology allows well-controlled layered stacking of antibodies and peroxidase enzymes on a micro-polymer scaffold, which avoids the occasional blocking of the antibody binding site during the labeling process. This technique results in a compact polymeric structure that easily penetrates to all cellular compartments, which provides superior sensitivity compared to other conventional HRP polymers with bulky dextran backbones. Plus, the system utilizes Fab' fragments of IgG secondary antibody instead of the whole IgG, which avoids the background caused by non-specific binding of whole IgG to endogenous Fc receptors.

#### Intended Use

For In-Vitro Diagnostic Use only. Super Sensitive Polymer-HRP Detection Systems are designed for the chromogenic detection of antigen-antibody binding reactions with mouse and/or rabbit IgG and IgM primary antibodies to achieve highly sensitive and specific immunohistochemical staining.

Name	Cat No	Pack Size	Components
Polymer Detection Kit, Universal (5 Components)	KPIHR001-5/50	50T	1) Peroxidase Block. 2) Anti-Mouse/Rabbit HRP-Polymer.
	KPIHR001-5/100	100T	
	KPIHR001-5/1000	1000T	3) 20X DAB Chromogen
			<ol> <li>DAB Substrate Buffer</li> </ol>
			5) Hematoxylin.
Polymer	KPIHR001-4/50	50T	<ol> <li>Peroxidase Block</li> <li>Anti-Mouse/Rabbit HRP-Polymer.</li> </ol>
Detection Kit,	KPIHR001-4/100	100T	
Universal	KPIHR001-4/1000	0 1000T	3) 20X DAB Chromogen
(4 Components)	KFINK001-4/1000	10001	<ol><li>DAB Substrate Buffer.</li></ol>
Polymer	KPIHR001-3/50	50T	<ul> <li>2) Anti-Mouse/Rabbit HRP-Polymer.</li> <li>3) 20X DAB Chromogen</li> <li>4) DAB Substrate Buffer.</li> </ul>
Detection Kit, Universal (3 Components)	KPIHR001-3/100	100T	
	KPIHR001-3/1000	1000T	

#### Recommended Instrument

Optical microscope (40X ~ 400X)

# Material & Component Not Provided

- Pipettes
- Immunohistochemical pap-pen
- Timer
- Drying box
- Incubation box
- Staining holder
- Coverslips
- Wash bottle.

### Handling/Storage

Cat No#KPIHR002, Ver1.0

Store at 2-8°C. Do not use after the printed expiration date.

#### **Reagent Preparation:**

Preparation: Use fresh biopsy or surgical tissue samples. Fix with 10% neutral buffered formalin for 8 to 24 hours. Follow pathological technical guidelines for sampling, dehydration, and paraffin embedding to form a paraffin block.

Storage: Store paraffin blocks in a dedicated, well-ventilated, dry cabinet. Blocks remain viable for 5 years at room temperature.

Slide Preparation: Spread tissue sections,  $3-5\mu$ m thick, onto adhesive slides. Absorb excess moisture with absorbent paper and gently pat on the slide rack. Bake the sections in an oven at 60°C (±5°C) for 30 to 60 minutes or let them sit overnight at 37°C.

Detection Timelines: For tissue slices at room temperature, complete detection within 7 days to ensure antigen distribution integrity. For slices stored at 2-8°C, complete detection within 3 months.

Preparation of Working Solution

Mix DAB substrate and DAB buffer solution in a 1:20 ratio. Prepare the DAB substrate working solution right before use.

Test Temperature Maintain between 18-25°C.

#### **Recommended Protocol**

- 1. Deparaffination: Immerse slides in xylene. Hydrate using a graded series of alcohols ending in water.
- 2. Peroxidase Block: Treat for 5 minutes with Peroxidase Blocking Reagents. Rinse twice using wash buffer for 5 minutes each.
- 3. Antigen Retrieval: Refer to the primary antibody data sheet for the recommended protocol.
- 4. Primary Antibody: Apply 2 drops (~100µl) or as needed of the primary antibody to cover the specimen. Incubate 30-60 minutes at room temperature (RT). Rinse twice using wash buffer for 5 minutes each.
- 5. Detection Polymer: Apply 2 drops (~100µl) or required amount of anti-Mouse/Rabbit HRP-Polymer. Incubate for 30 minutes at RT. Rinse twice with wash buffer for 5 minutes each.
- 6. DAB Chromogen: Mix DAB Chromogen with DAB Substrate Buffer in a 1:20 ratio. Typically, 100µl is sufficient for one slide. Incubate slides for 5 minutes at RT, then rinse with deionized water.
- 7. Counterstain: Use hematoxylin and rinse with deionized water.
- 8. Dehydration & Sealing: Successively immerse slides in 70%, 85%, and 95% ethanol for 2 minutes each. Soak in anhydrous ethanol twice for 2 minutes each time. Transparentize with xylene twice, 2 minutes each time. Seal with neutral balata and a coverslip.
- 9. Observation: Examine under an optical microscope.

#### Quality Control

Positive Control: A positive control ensures proper tissue preparation and staining. It should be included in every test for comparison. These controls monitor the accuracy of steps and reagents but aren't for definitive diagnosis. If a positive tissue control fails to stain appropriately, deem the test sample invalid.

Blank Control: Every staining should have a blank control reagent for comparison. Use this instead of the antibody to determine non-specific staining and enhance interpretation of specific antigen site staining.

#### **Results Interpretation**

The staining results must be based on the positive and negative control experiments:

Positive: the target antigen site shows brown staining.

Negative: no brown staining.

Results Interpretation should be determined by a qualified pathologist.

#### Test Limitations

- 1. Expertise Required: Immunohistochemical pathology diagnosis involves multiple steps. Ensuring accuracy requires rigorous training in reagent selection, sample preparation, and staining interpretation. Standardization is best achieved through professional operators and accredited labs, minimizing variations in staining.
- Tissue Processing Impact: The preparatory stages significantlyinfluence staining outcomes. Mistakes in fixation, handling (e.g., freezing, thawing, washing, drying, slicing), or contamination can lead to false results or abnormal staining. Variations in fixation and embedding methods can also affect results.
- 3. Counter Staining Precision: Both excessive and insufficient counter staining can distort results interpretation.
- 4. Comprehensive Evaluation: Interpreting staining, whether positive, negative, or absent, should consider clinical history, cell morphology, and other histopathological context. This interpretation must be complemented by morphological studies, appropriate controls, and other diagnostic tests. A pathologist should integrate these findings with clinical conditions and other examinations.
- 5. Reagent Reactions: There's potential for unexpected reactions when using reagents on untested tissues. Due to the inherent variability in antigen expression across tumors or pathological tissues, unforeseen reactions may occur.
- 6. False Positives: Non-immunological protein bindings or substrate reaction products can lead to false positive results. Red blood cells and cytochrome C can also be sources of errors.
- 7. Kit Limitations: This kit has been validated only for tissues fixed with 10% neutral buffered formalin and paraffin-embedded. It is not suitable for other specimen types or uses, such as flow cytometry.

#### Cautions

- 1. Preparation: Carefully review the instruction for use prior to experimentation.
- 2. Professional Use: Only trained professionals should use the reagent within its validity period. Do not use if leakage, contamination, or deterioration is observed.
- 3. Compatibility: Mixing components with products from other manufacturers may result in abnormal staining.
- 4. Safety Precautions: Avoid skin, eye contact, or inhalation. In case of contact, promptly rinse the affected area with ample water.
- 5. Control Slides: Every staining must include both a positive contrast slide and a blank control. Without these controls, results are inadmissible.
- 6. Procedure Adherence: Incorrect antigen retrieval, incubation duration, temperature, or other methods can skew results.
- 7. Sample Freshness: Samples stored at room temperature should be processed within 7 days to prevent antigen degeneration and potential false negatives.
- 8. Control Indications: If a positive control fails to stain correctly, this suggests procedural error, rendering the sample batch results invalid.
- 9. DAB Safety: Given DAB's potential mutagenicity, ensure safety precautions during use. Waste disposal should adhere to relevant legal requirements.
- 10. Kit Limitations: The efficacy of this kit on non-formalin fixed tissues remains unverified.

**Note:** The packaging of this product contains natural rubber latex which may cause allergic reactions.

#### Troubleshooting

Refer to the troubleshooting section in the KRISHGEN Detection Kits user manual for remedial actions on detection system related issues, or contact KRISHGEN Technical Support Department at 1-800-421-4149 or support@KRISHGEN.com or your local distributor to report unusual staining.

#### Precautions

Specimens and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Avoid microbial contamination of reagents to minimize non-specific staining. Wear suitable Personal Protective Equipment. Never pipette reagents by mouth. Avoid contact of reagents and specimens with skin and mucous membranes. If reagents or specimens come into contact with sensitive area, wash with copious amounts of water.

Some reagents in this kit contain sodium azide at concentrations of less than 0.1%. Sodium azide is not classified as a hazardous chemical at these concentrations, but proper handling protocols should be observed. DAB is classified as a possible carcinogen and can cause skin irritation upon contact. For more information on product hazards, precautions and waste disposal, Material Safety Data Sheets are available upon request. Dispose of unused reagents according to Local, State and Federal Regulations.

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