HiDef Detection™ Alk Phos Mouse/Rabbit Polymer System

For In Vitro Diagnostic Use (IVD)
Instructions for use

Catalog No. **962D-20 – 50 ml Kit**
962D-30 – 100 ml Kit

Intended Use
The HiDef Detection™ Alk Phos Mouse/Rabbit Polymer System is intended for laboratory use to indentify by light microscopy target antigens in formalin fixed paraffin embedded tissue or frozen tissue when used in conjunction with antibodies and appropriate chromogen in the IHC staining process.

Cell Marque offers and recommends the use of its primary antibodies and appropriate chromogen.

Summary and Explanation
HiDef Detection™ Alk Phos Mouse/Rabbit Polymer System is an extremely sensitive immunoenzymatic detection kit. The kit is an indirect, biotin-free polymer detection kit for detecting mouse IgG, mouse IgM and rabbit primary antibodies. As a result, nonspecific staining from endogenous avidin-biotin activity is eliminated.

Principles and Procedure
The primary antibody specific to an antigen on the tissue section is detected by HiDef Amplifier followed by HiDef polymer step. The antigen sites are then intensely visualized with an appropriate substrate/ chromogen.

Refer to the Protocol Recommendation section for recommended use.

Materials and Methods

Reagents supplied as ready-to-use components in dropping bottles:

Kit Cat. No	Reagent Cat. No.	Contents	Vol. (ml)
962D-20 50 ml kit	962D-21	HiDef Amplifier for Mouse and Rabbit	50
	962D-22	HiDef Alk-Phos Polymer Detector	50
962D-30 100 ml kit	962D-31	HiDef Amplifier for Mouse and Rabbit	100
	962D-32	HiDef Alk-Phos Polymer Detector	100

Materials Reagents Needed But Not Provided:

Microscope slides, positively charged
Drying Oven
Positive and Negative Control
Clearing Agent (Xylene, Clearene, etc.)
Ethanol or reagent Alcohol
Pressure Cooker*
Wash Buffer*
Distilled Water
Pretreatment Reagents*
Enzyme Digestion*
Primary antibody*
Negative Control Reagents*
Chromogen*
Hematoxylin*
Mounting Medium*

**See Cell Marque Catalog for product numbers. Some of the reagents listed are based on specific applications and detection system used.*

Storage and Stability
The HiDef Detection™ Alk Phos Mouse/Rabbit Polymer System should be stored at 2-8° C and protected from light.

Do not use after expiration date printed on vials.

Recommended Protocol Instructions
1. Cut tissue sections approximately 3 microns and dry completely.
2. Deparaffinize, rehydrate, and epitope retrieve, following recommended pretreatment protocol for each antibody.
3. Wash with 5 changes of wash buffer.
4. Cover tissue with primary antibody following manufacturer's recommended protocol.
5. Wash with 3 changes of wash buffer.
6. Apply HiDef Amplifier and incubate for 10 minutes at room temperature.
7. Rinse with 3 changes of wash buffer.
8. Apply HiDef Alk-Phos Polymer and incubate for 10 minutes at room temperature.
9. Rinse with 3 changes of wash buffer.
10. Cover tissue with chromogen; incubate for 5 minutes to 20 minutes at room temperature as necessary to allow for proper color development.
11. Rinse slides in DI water; counterstain and dehydrate.
12. Coverslip

Protocol Notes
1. Cell Marque offers and recommends the use of Permanent Red Chromogen Kit (956D series); optimal results may be obtained by developing this chromogen in the dark.
2. Do not use PBS buffer as phosphates act as a competitive inhibitor to alkaline phosphatase enzyme. Use TBS Wash Buffer.
3. Incubate slide with 10% goat serum for 10-15 minutes if necessary to reduce nonspecific background staining due to nonspecific interaction between the tissue antigen and the primary antibody or further titrate primary antibody.

Interpretation of Results
The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

Quality Control
Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on tissue controls.

Warning/Precautions
Refer to product MSDS

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Limitations & Warranty
Immunohistochemistry is a multiple step diagnostic process that requires specialized training and selection of appropriate reagents and controls. The protocols for a specific application can vary. It is the responsibility of the end user to determine optimal conditions.

There are no express or implied warranties which extend beyond this datasheet. Cell Marque is not liable for personal injury, property damage, or economic loss caused by this product.

Troubleshooting
Refer to reagent-specific protocol recommendation according to data sheet provided.

For further help, feel free to contact Cell Marque's Technical Support at +1-800-665-7284.

References
1. NCCLS. Quality Assurance for Immunocytochemistry: Approved Guideline. CLSI document MM4-A- (ISBN 1-56238-396-5). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999
2. Bisgaard K, Pluzek KP: Use of polymer conjugates in immunohistochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates. Abstract XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungary, October 20-25, 1996.
3. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.
4. Shi ZR, Au A, Soriano R et al: Non biton Amplification (NBA) kit prevents nonspecific background staining of endogenous biton induced by heat epitope retrieval (HIER) procedure. The J Histotechnol 23:327, 2000.