

HiDef Detection™ HRP Polymer System

For In Vitro Diagnostic Use (IVD)
Instructions for use

INTENDED USE

HiDef Detection™ HRP Polymer System is intended for use in immunohistochemistry (IHC) staining protocols. This reagent is designed to detect target antigens in formalin-fixed, paraffin-embedded tissue sections when used in conjunction with antibodies and appropriate chromogen in the IHC staining process.

SUMMARY AND EXPLANATION

The HiDef Detection™ Polymer System is a visualization system that is ready-to-use in immunohistochemical protocols. This two-step system uses an indirect method resulting in an antibody-enzyme complex that universally detects mouse and rabbit primary antibodies. The resulting chromogenic reaction can be visualized by HRP-compatible chromogens using light microscopy. It is biotin-free and eliminates non-specific staining that could result from endogenous biotin. This visualization system consists of two detection reagents and is based on the sequential application of HiDef Detection™ Amplifier (Mouse and Rabbit) followed by HiDef Detection™ HRP Polymer Detector, amplifying the detection of low expressing antigens. This detection system is compatible with both manual and open automated staining platforms.

PRINCIPLES AND PROCEDURES

The primary antibody specific to an antigen on the tissue section is detected by HiDef Detection™ Amplifier (Mouse & Rabbit) followed by HiDef Detection™ Polymer Detector step. The antigen sites are then visualized with an appropriate substrate/chromogen.

Cell Marque offers and recommends the use of its primary antibodies and appropriate chromogen with this detection kit.

MATERIALS AND METHODS

Kit Cat. No.	Reagent Cat. No.	Contents	Vol. (ml)
954D-10 7 ml kit	954D-11	HiDef Detection™ Amplifier (Mouse & Rabbit)	7 ml
	954D-12	HiDef Detection™ Polymer Detector	7 ml
954D-20 50 ml kit	954D-21	HiDef Detection™ Amplifier (Mouse & Rabbit)	50 ml
	954D-22	HiDef Detection™ Polymer Detector	50 ml

954D-30 100 ml kit	954D-31	HiDef Detection™ Amplifier (Mouse & Rabbit)	100 ml
	954D-32	HiDef Detection™ Polymer Detector	100 ml
954D-40 1000 ml kit	954D-41	HiDef Detection™ Amplifier (Mouse & Rabbit)	1000 ml
	954D-42	HiDef Detection™ Polymer Detector	1000 ml
954D-50 15 ml kit	954D-51	HiDef Detection™ Amplifier (Mouse & Rabbit)	15 ml
	954D-52	HiDef Detection™ Polymer Detector	15 ml

Materials and Reagents Needed But Not Provided

- TBS or PBS wash buffer*
- Volumetric flask/graduated cylinder
- Microscope slides, positively charged
- Drying oven
- Positive and negative controls
- Clearing agent (xylene, Clearene, etc.)
- Ethanol or reagent alcohol
- Slide rack*
- Staining dishes*
- Pressure cooker*
- Pretreatment reagents*
- Proteolytic enzyme
- Peroxidase block
- Negative control reagents*
- 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

Store at 2-8° C up to a minimum of 24 months from the date of manufacture (see product label for expiration date). Keep protected from light.

Reagent Preparation

This product contains Ready-to-Use reagents.

Recommended Protocol Instructions

- Cut tissue sections approximately 3 microns and dry completely.
- Deparaffinize and rehydrate tissue section.
- Where required, perform pretreatment with Heat Induced Epitope Retrieval (HIER) or protease according to manufacturer's recommendations; for most Cell Marque antibodies, the preferred method is the use of Heat Induced Epitope Retrieval (HIER) techniques using Cell Marque's EDTA based retrieval solution Trilogy™ in conjunction with a pressure cooker for 15 minutes at 120°C. The preferred method allows for simultaneous deparaffinization, rehydration, and epitope retrieval. Upon completion, rinse with distilled or deionized water.

- Place slides in peroxidase block for 10 minutes; rinse with IHC wash buffer.
- Apply primary antibody according to manufacturer's recommended protocol; rinse with IHC wash buffer.
- Apply HiDef Detection™ Amplifier for 10 minutes at room temperature; rinse with IHC wash buffer.
- Apply HiDef Detection™ HRP Polymer Detector for 10 minutes at room temperature; rinse with IHC wash buffer.
- Apply HRP-compatible chromogen according to manufacturer's recommendations; rinse with distilled or deionized water.
- Counterstain and coverslip.

Protocol Notes

N/A

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 PROCEDURES

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

WARNINGS AND PRECAUTIONS

- This product is for *in vitro* diagnostic use by professionals only.
- Do not use after expiration date printed on product labels. The user must validate any storage conditions other than those specified in the package insert.
- Bring all reagents, slides, and specimens to room temperature (18-24° C) prior to use.
- Cross contamination of reagents or samples may give false results.
- Avoid microbial contamination of reagents, as this could produce incorrect results.
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Do not smoke, eat, or drink in areas where specimens or reagents are handled.
- Avoid splashing or generation of aerosols at all times.
- Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use.

- Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
- Refer to product MSDS
- Do not freeze.
- Color variation is only cosmetic and does not affect performance.

LIMITATIONS AND 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.

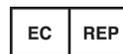
TROUBLESHOOTING

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

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

DISCLAIMERSwww.cellmarque.comEMERGO EUROPE
Molenstraat 15, 2513 BH, The Hague, NL.

CM Template #1.0