Permanent Red Chromogen Kit

For In Vitro Diagnostic Use (IVD)
Instructions for use

Catalog No. 960D-10 – 30 ml Kit
960D-20 – 100 ml Kit

Intended Use
The permanent Red Chromogen kit 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 detection reagents using Alkaline Phosphatase enzyme in the IHC staining process.

Cell Marque offers and recommends the use of its Alkaline Phosphatase labeled detection systems.

Summary and Explanation
When in the presence of Alkaline Phosphatase, Permanent Red Chromogen produces a non-fading, bright red precipitate that can be readily visualized using light microscopy. The precipitate is insoluble in organic solvents and can be coverslipped with permanent mounting media.

Principles and Procedure
The chromogen is the final step in the detection portion of the IHC process; it enables the antibody-antigen complex to be viewed under light microscope. Permanent Red acts as an electron donor in the presence of enzyme Alkaline Phosphatase. As a result, Permanent Red gets reduced and the color change occurs.

Refer to the Protocol Recommendation section for recommended use.

Materials and Methods

Reagents Supplied As:

Kit Cat. No	Reagent Cat. No.	Contents	Vol. (ml)
960D-10 30 ml kit	960D-11	Perm. Red Reagent 1	0.7
	960D-12	Perm. Red Reagent 2	0.7
	960D-13	Perm. Red Reagent 3	0.7
	960D-14	Perm. Red Buffer	30.0
960D-20 100 ml kit	960D-21	Perm. Red Reagent 1	2.25
	960D-22	Perm. Red Reagent 2	2.25
	960D-23	Perm. Red Reagent 3	2.25
	960D-24	Perm. Red Buffer	100.0

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*
Avidin-Biotin block*
Peroxidase Block
Primary antibody*
Negative Control Reagents*
Detection Kits*
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 Permanent Red Chromogen Kit should be stored at 2-8^{0}C and protected from light.

Do not use after expiration date printed on vials.

Recommended Protocol Instructions
1. Prepare the working solution as follows:
   1.1 To 5 ml of Permanent Red Buffer:
   1.2 Add 2 Drops of Reagent 1. Mix Well.
   1.3 Add 2 Drops of Reagent 2. Mix Well.
   1.4 Add 2 Drops of Reagent 3. Mix Well.
2. Incubate tissue with working solution at room temperature for 15-30 minutes.
3. Rinse tissue with distilled water.
4. Counterstain with hematoxylin.
5. Dehydrate and coverslip with permanent mounting media.

Protocol Notes:
1. Use the working solution within 15 minutes of preparation or decreased sensitivity may result.
2. Do not use PBS buffer as phosphates act as a competitive inhibitor to alkaline Phosphatase enzyme. Use TBS Wash Buffer.
3. Improving staining may be obtained by developing the substrate in the dark.
4. Endogenous Alkaline Phosphatase activity can be inhibited by the addition of levamisole to the Permanent Red Buffer prior to the preparation of the working solution. Intestinal Alkaline Phosphatase can be inhibited with several tissue pretreatments.

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Interpretation of Results
The Cell Marque Permanent Red Kit causes a bright red reaction product to precipitate at the antigen sites localized by the primary antibody. A qualified pathologist must evaluate controls and qualify the stained product before interpreting results.

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

Little is known about the toxicity and carcinogenicity of the Permanent Red Kit components. Care should be taken in the handling and disposing of all the reagents.

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 expressed 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. Journal of Clinical Pathology Vol. 34, Bulman AS and Hedyerman E; pgs. 1349-1351. USA 1981.
3. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.