

PermaGreen™/HRP

K074
K074-110

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

PermaGreen™/HRP is a substrate-chromogen system designed to be used for either IHC or ISH when utilizing horseradish peroxidase. PermaGreen™/HRP can be permanently mounted to produce a bright green color that can be easily distinguished from other stains.

Principles of the Procedures

Substrate/chromogen in conjunction with horseradish peroxidase (HRP)-based immunostaining or *in situ* hybridization systems. With at least 4 hour working stability, this substrate/chromogen system can be used in any automation system as well as manual use.

Reagents Provided

Kit Contents	30 mL	110 mL
PermaGreen™/HRP Substrate Buffer	30 mL	110 mL
PermaGreen™/ HRP Chromogen	1 mL	3 mL
Empty Mixing Bottle	1	1

Prepare the Following Solutions Before Use

1. Aliquot 1mL of PermaGreen™/HRP Substrate Buffer in a mixing bottle.
2. Add one drop (~20µl) of concentrated PermaGreen™/HRP Chromogen solution.
3. Replace tip, mix, and allow solution to reach room temperature before using.
4. The PermaGreen™/HRP chromogen-substrate working solution is light sensitive and should be kept away from light as much as possible.
5. Working solution is stable for up to 4 hour, for optimal results prepare fresh reagent.

Materials Required But Not Provided

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com.

Storage and Handling

Store at 2°-8°C away from light. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure

- 1) Once sections have been incubated with peroxidase, wash sections with wash buffer then follow protocol of choice:
 - a. **Pre-Mix Working Solution: (Automation)**
PermaGreen™/HRP has a working solution stability of at least 4 hour and can be loaded directly onto instrument as a single solution. Reduce exposure to light to achieve optimal staining. Working solution is applied directly to slide. Incubate for 5-10 min.
 - b. **On Board Mixing: (Automation)** Instruments that have on-board mixing capability can load the chromogen and substrate-buffer components independently. Working solution is made mixing reagents 1:50 in on-board mixing station before application to slide. Incubate for 5-10 min.
 - c. **Manual Use:** Mix substrate-chromogen and buffer in a 1:50 ratio and apply directly to slide. Incubate for 5-10 min.
- 2) Counterstain with Hematoxylin or other counterstain. Wash with DI water followed by immuno wash buffer.
- 3) Mounting: Slides should be air dried (do not dehydrate in alcohol or xylene). After rinsing off counterstain in DI H₂O, leave slides on benchtop for at least 20 minutes to air dry, and then permanently mount.

Recommendation:

For best color preservation and long term slide storage, we recommend using ImmunoHisto-Sealer (**K076**) after counterstaining.

Precautions

- 4) Consult local and/or state authorities with regard to recommended method of disposal.
- 5) Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
- 6) Avoid microbial contamination of reagents. Contamination could produce erroneous results.
- 7) This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
- 8) If reagent contacts these areas, rinse with copious amounts of water.
- 9) Do not ingest or inhale any reagents.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.