ab209101
Rabbit specific IHC polymer detection kit HRP/DAB

Instructions for use:

For detection of rabbit antibodies bound to antigens in tissue sections using light microscopy.

This product is for research use only and is not intended for diagnostic use.

Version 1c Last Updated 24 September 2015

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1. BACKGROUND

Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) is a sensitive immunoenzymatic system for detecting antibodies bound to antigens within tissue sections by light microscopy. Signal amplification is achieved using an indirect polymer detection system. As Rabbit specific IHC polymer detection kit HRP/DAB does not use avidin or biotin, nonspecific staining from endogenous avidin-biotin activity is eliminated and there is no need for additional steps to block endogenous biotin. The primary antibody specific to an antigen on the tissue section is detected by primary antibody Amplifier. The HRP polymer is added to enable detection with signal amplification. Finally, antibody-bound antigen sites are visualized by adding an appropriate substrate/chromogen (not provided).

2. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

3. STORAGE AND STABILITY

Store kit at 4ºC upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

4. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Amplifier	15 mL	4°C
Detector	15 mL	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Mounting media (ab64230)
- Glass Slides
- Cover Slips
- Slide Rack
- Coplin Jar
- H_2O_2
- Goat serum (ab7481)
- PBS (ab64026)
- Primary antibody
- DAB chromogen (ab64238)
- Counterstains

7. TECHNICAL HINTS

- Avoid cross contamination of samples or reagents by changing tips between reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

8. REAGENT PREPARATION

8.1. **Amplifier**

Ready to use as supplier. Store at 4°C

8.2. **Detector**

Ready to use as supplied. Store at 4°C

9. STAINING PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**

9.1. Deparaffinize and rehydrate tissue section. Please see www.abcam.com/protocols/ihc-deparaffinization-protocol for an example protocol.

9.2. Incubate slide in diluted H_2O_2 (e.g., 0.3% H_2O_2) for 10-15 minutes if necessary to reduce nonspecific background staining due to endogenous peroxidase.

9.3. Wash 3 times with tap water.

9.4. If required, incubate slide with 10% goat serum for 10-15 minutes to reduce non-specific background staining arising from non-specific interactions between the tissue and the primary antibody or further titrate the primary antibody.

9.5. Apply primary antibody and incubate according to manufacturer's protocol.

9.6. Wash 3 times in PBS.

9.7. Apply Amplifier and incubate for 10 minutes at RT.

9.8. Wash 3 times in PBS.

9.9. Apply Detector and incubate for 10 minutes at RT.

9.10. Wash 3 times in PBS.

9.11. Apply chromogen (DAB) to the tissue and incubate for 5-10 minutes, depending on the desired stain intensity.

9.12. Counterstain and mount coverslip using mounting media.

10. NOTES

RESOURCES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

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