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ab236466 - Mouse and Rabbit Specific HRP/DAB IHC Detection Kit - Micro-polymer

For the detection of a specific antibody bound to an antigen in tissue sections.

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

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1. Overview

ab236466 is a biotin free immunoenzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated rabbit or mouse primary antibody specific to the target antigen, a secondary antibody-Goat anti-rabbit HRP Conjugate and substrate-chromogen (DAB).

2. Principle of Assay

This detection system detects a specific antibody bound to an antigen in tissue sections. The specific antibody is located by a secondary antibody polymerized to an enzyme. The specific antibody, secondary antibody -enzyme complex is then visualized with an appropriate substrate/chromogen. The advantage offered by a micro-polymer detection system over an ABC based detection system is that it is biotin free (ideal for studying tissue rich in endogenous biotin e.g. kidney or brain tissue). In addition, the use of a micro-polymer detection system is advantageous over a polymer detection system as a smaller detection complex is formed rather than a polymer backbone aiding better tissue penetration.

3. 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 handled 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.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Do not freeze. 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.

5. Limitations

- Assay 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.

6. Materials Supplied

Item	Quantity (15 ml)	Quantity (60 ml)	Quantity (125 ml)	Storage Condition
Protein Block	1 x 15 mL	1 x 60 mL	1 x 125 mL	4°C
Mouse Specifying Reagent (Complement)	1 x 15 mL	1 x 60 mL	1 x 125 mL	4°C
50X DAB Chromogen	1 x 0.5 mL	1 x 2 mL	1 x 4 mL	4°C
Hydrogen Peroxide Block	1 x 15 mL	1 x 60 mL	1 x 125 mL	4°C
DAB Substrate	1 x 15 mL	1 x 60 mL	1 x 125 mL	4°C
Goat anti-rabbit HRP-Conjugate	1 x 15 mL	1 x 60 mL	1 x 125 mL	4°C

7. Materials Required, Not Supplied

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

- Primary antibody

8. Technical Hints

- Make sure all buffers and solutions are at room temperature before starting the experiment.
- The inclusion of negative controls will aid in accurate interpretation of the staining results and help in determining false positives. Negative control fixed and processed in the same manner as the tissue specimen placed on every slide run, during manual or automated staining, in addition to the target tissue is strongly recommended. For the test to be considered valid, the negative control should be clean. This negative tissue control should be included to ensure that the other treatment procedures did not create false positive staining.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The reagents must be returned to the storage conditions (4°C) immediately after use.

9.1 Protein Block

Ready to use as supplied.

9.2 Mouse Specifying Reagent (Complement)

Ready to use as supplied.

9.3 50X DAB Chromogen

Ready to use as supplied.

9.4 Hydrogen Peroxide Block

Ready to use as supplied.

9.5 DAB Substrate

Ready to use as supplied.

9.6 Goat anti-rabbit HRP-Conjugate

Ready to use as supplied.

10. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature prior to use.

10.1 Deparaffinize and rehydrate formalin-fixed paraffin-embedded tissue section.

10.2 Add enough drops of Hydrogen Peroxide Block to cover the sections. Incubate for 10 minutes. Wash 2 times in buffer.

10.3 Perform appropriate pretreatment if required. Wash 3 times in buffer.

10.4 Apply Protein Block (if required) and incubate for 10 minutes at room temperature to block nonspecific background staining. Wash 1 time in buffer.

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

10.6 Wash 3 times in buffer.

10.7 Apply Mouse Specifying Reagent (Complement) and incubate for 10 minutes at room temperature. Wash 2 times in buffer.
Δ NOTE: The Complement reagent is a Rabbit anti-mouse secondary that allows the Goat anti-rabbit HRP conjugate to detect the mouse primary. The Complement step is only necessary if you are using a mouse primary antibody.

10.8 Apply Goat anti-rabbit HRP-conjugate and incubate for 15 minutes. Wash 4 times in buffer.

10.9 Add 30 µl (1 drop) DAB Chromogen to 1.5 ml (50 drops) of DAB Substrate, mix by swirling and apply to tissue. Incubate for 1-10 minutes. Rinse 4 times in buffer.

10.10 Apply counterstain according to manufacturer's instructions.

10.11 Dehydrate if required and coverslip.

Δ Note: The specificity and sensitivity of antigen detection is dependent on the specific primary antibody used.

Please contact our Technical Support team for more information.

11. Troubleshooting

Problem	Reason	Solution
No Staining	The primary antibody and the secondary antibody are not compatible.	Use secondary antibody that was raised against the species in which the primary was raised (e.g. primary is raised in rabbit, use anti-rabbit secondary).
	Not enough primary antibody is bound to the protein of interest.	Use less dilute antibody, Incubate longer (e.g. overnight) at 4°C.
	The antibody may not be suitable for IHC procedures which reveal the protein in its native (3D form).	Test the antibody in a native (non-denatured) WB to make sure it is not damaged.
	The protein is not present in the tissue of interest.	Run a positive control recommended by the supplier of the antibody.
	Primary or secondary antibody/amplification kit may have lost its activity due to improper storage, improper dilution or extensive freezing/thawing.	Run positive controls to ensure that the primary/secondary antibody is working properly.
	The protein of interest is not abundantly present in the tissue.	Use an amplification step to maximize the signal.

No Staining	The secondary antibody was not stored in the dark.	Always prevent the secondary antibody from exposure to light.
	Deparaffinization may be insufficient.	Deparaffinize sections longer, change the xylene.
	Fixation procedures (using formalin and paraformaldehyde fixatives) may be modifying the epitope the antibody recognizes.	Use antigen retrieval methods to unmask the epitope, fix for less time.
	The protein is located in the nucleus and the antibody (nuclear protein) cannot penetrate the nucleus.	Add a permeabilizing agent to the blocking buffer and antibody dilution buffer.
	The PBS buffer is contaminated with bacteria that damage the phosphate groups on the protein of interest.	Add 0.01% azide in the PBS antibody storage buffer or use fresh sterile PBS.

Problem	Reason	Solution
High Background	Blocking of non-specific binding might be absent or insufficient.	Increase the blocking incubation period and consider changing blocking agent. Abcam recommends 10% normal serum 1hr for sections or 1-5% BSA for 30 min for cells in culture.
	Incubation temperature may be too high.	Incubate sections or cells at 4°C.
	The primary antibody concentration may be too high.	Titrate the antibody to the optimal concentration, incubate for longer but in more dilute antibody (a slow but targeted binding is best).
	The secondary antibody may be binding non-specifically (damaged).	Run a secondary control without primary antibody.
	Tissue not washed enough, fixative still present.	Wash extensively in PBS between all steps.
	Endogenous peroxidases are active.	Use enzyme inhibitors i.e. Levamisol (2 mM) for alkaline phosphatase or H_2O_2 (0.3% v/v) for peroxidase.

High Background	Fixation procedures (using formalin and paraformaldehyde fixatives) are too strong and modified the epitope the antibody recognizes.	Change antigen retrieval method, decrease the incubation time with the antigen unmasking solution.
	Too much amplification (amplification technique).	Reduce amplification incubation time and dilute the amplification kit.
	Too much substrate was applied (enzymatic detection).	Reduce substrate incubation time.
	The chromogen reacts with the PBS present in the cells/tissue (enzymatic detection).	Use Tris buffer to wash sections prior to incubating with the substrate, then wash sections/cells in Tris buffer.
	Permeabilization has damaged the membrane and removed the membrane protein (membrane protein).	Remove permeabilizing agent from your buffers.

Problem	Reason	Solution
Non-specific staining	Primary/secondary antibody concentration may be too high.	Try decreasing the antibody concentration and/or the incubation period. Compare signal intensity against cells that do not express the target.
	Endogenous peroxidases are active.	Use enzyme inhibitors i.e. Levamisol (2 mM) for alkaline phosphatase or H_2O_2 (0.3% v/v) for peroxidase.
	The primary antibody is raised against the same species as the tissue stained (e.g. mouse primary antibody tested on mouse tissue). When the secondary antibody is applied it binds to all the tissue as it is raised against that species.	Use a primary antibody raised against a different species than your tissue.
	The sections/cells have dried out.	Keep sections/cells at high humidity and do not let them dry out.

12. Notes

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