ab64259 - Mouse Specific HRP/DAB Detection IHC Kit

Instructions for Use

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. Introduction

ab64260 is a labeled streptavidin-biotin immunoenzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody that reacts with the primary antibody, enzyme-labeled streptavidin, and substrate-chromogen.

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 biotin-conjugated secondary antibody. This step is followed by the addition of a streptavidin-enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody, secondary antibody, and streptavidin-enzyme complex is then visualized with an appropriate substrate/chromogen.

3. Kit Contents

Item	Quantity
Protein Block	15 mL
Biotinylated Goat Anti-Mouse	15 mL
50x DAB Chromogen	0.5 mL
Hydrogen Peroxide Block	15 mL
DAB Substrate	15 mL
Streptavidin Peroxidase	15 mL

4. Storage and Handling

Store at 2-8°C. Do not freeze. The reagents must be returned to the storage conditions identified above immediately after use.

5. Additional Materials Required

- Primary antibody

6. Protocol

All steps are performed at room temperature.

Note: 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.

Staining Protocol

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

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

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

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

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

6. Wash 4 times in buffer. Apply Biotinylated Goat Anti-Mouse and incubate for 10 minutes at room temperature. Wash 4 times in buffer.

7. Apply Streptavidin Peroxidase and incubate for 10 minutes at room temperature.

8. Rinse 4 times in buffer. 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.

9. Apply counterstain according to manufacturer's instructions.

10. Dehydrate if required and coverslip.

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

7. General IHC Troubleshooting Tips

Problem	Cause	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.

	The primary/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.
	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	**Cause**	**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.

	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.

	Pemeabilization has damaged the membrane and removed the membrane protein (membrane protein).	Remove permeabilizing agent from your buffers.

Problem	**Cause**	**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.

Find more tips and reagents tailored for your IHC protocols at:

www.abcam.com/IHC

Technical Support

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