ab64238 - DAB Substrate Kit

For the immunohistochemical staining

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

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.
- 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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

Storage and Stability: Store kit at +4°C immediately upon receipt.

Materials Supplied:

Item	Quantity (60 mL)	Quantity (125 mL)
DAB Chromogen (50X)	2 mL	4 mL
DAB Substrate	60 mL	125 mL

Materials Required, Not Supplied

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

- Primary antibody
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Staining Protocol: Equilibrate all materials and prepared reagents to room temperature prior to use.

ΔNOTE: 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.

1. Add 30 µl (1 drop) DAB Chromogen to 1.5 ml (50 drops) of DAB Substrate, mix by swirling and apply to tissue.
2. Incubate for 1-10 minutes. Rinse 4 times in buffer.
3. Apply counterstain according to manufacturer's instructions.
4. Dehydrate if required and coverslip.

Troubleshooting

No staining

Reason	Solution
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.
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.
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 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.

High background

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

Non-specific staining

Reason	Solution
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.
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.

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)