

DAB Substrate Kit ab64238

★★★★★ [7 Abreviews](#) [305 References](#) [3 Images](#)

Overview

Product name DAB Substrate Kit

Product overview DAB substrate kit ab64238 is used for IHC staining using HRP / peroxidase enzyme for detection. Reagent is stable for up to 6 hours after mixing the two components.

DAB (3,3'Diaminobenzidine) is the most commonly used chromogen for immunohistochemical staining. In the presence of HRP / peroxidase, DAB produces a brown precipitate that is insoluble in alcohol.

This product is a two component form consisting of a liquid, refrigerator stable DAB Chromogen and DAB Substrate.

IHC protocol suitable for use with DAB substrate kit:

For frozen sections, skip steps 1 and 2.

1. Deparaffinize and rehydrate formalin-fixed paraffin-embedded tissue section.
2. Use appropriate **antigen retrieval buffer or enzyme** (primary antibody dependent) to treat sections. Wash 3 times in buffer.
3. Add enough **hydrogen peroxide blocking solution** to cover the sections. Incubate for 10 minutes. Wash 2 times in buffer. If necessary, use **avidin biotin blocking**.
4. Apply **protein block** (or **normal serum** from same species as secondary antibody) and incubate for 5 minutes at room temperature to block nonspecific background staining. Wash once in buffer.
5. Apply primary antibody in **antibody diluent** and incubate.
6. Wash 4 times in buffer. Incubate slide with **biotinylated secondary antibody** (or **HRP polymer secondary antibody** and skip step 7). Wash 4 times in buffer.
7. Apply **streptavidin-HRP** and incubate for 10 minutes at room temperature.

8. Rinse 4 times in buffer. **Place slide in DAB substrate ab64238 or AEC substrate and incubate until desired color is achieved (1-10 mins).** Rinse 4 times in buffer.

9. Add enough drops of **hematoxylin** to cover the section. Incubate for 1 minute.

10. Rinse 7-8 times in tap water. Add **mounting medium** to cover the section.

Notes

Find complete IHC kits, and reagents for antigen retrieval, blocking, signal amplification, visualization, counterstaining, and mounting in the **IHC kits and reagents guide**.

Tested applications

Suitable for: IHC-P

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	60 ml	125 ml
50x DAB Chromogen	1 x 2ml	1 x 4ml
DAB substrate	1 x 60ml	1 x 125ml

Relevance

3,3' Diaminobenzidine (DAB) is a widely used chromogen for immunohistochemical staining. In the presence of peroxidase enzyme, DAB produces a brown precipitate that is insoluble in alcohol.

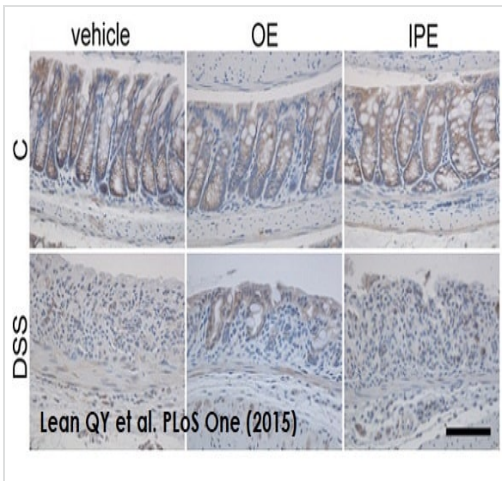
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab64238 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use at an assay dependent dilution.

Images



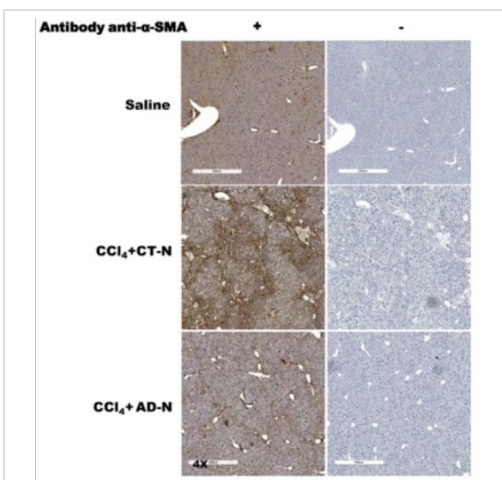
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)

Lean QY et al. PLoS One (2015) Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

Immunohistochemical analysis of healthy and colitis mouse colon sections (untreated and treated with enoxaparin) labeling claudin-4 with **ab15104** at 1/200. **ab7090**, anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (HRP) at 1/300 was used as the secondary antibody. ab64238 at 1/50 was used to develop the histological signal.

Antigen retrieval was performed by incubating the sections for 10 minutes at 97°C in 1 mM EDTA buffer, pH 8.0 or 10 mM citrate buffer, pH 6.0.

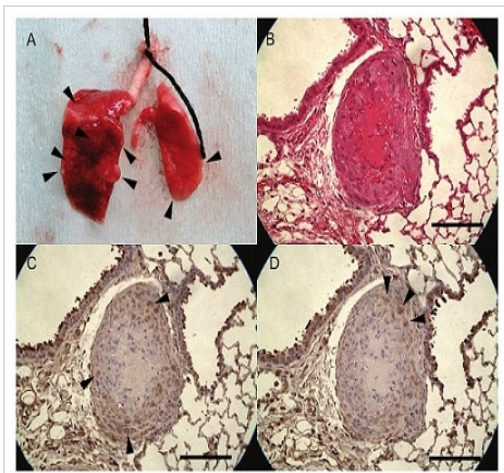
Control, C; untreated colitis, DSS; oral enoxaparin, OE; intraperitoneal injection of enoxaparin, IPE.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)

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Immuno-staining of formalin-fixed and paraffin-embedded liver sections with ab64238. Sections were obtained from control (saline) and treated with CCl₄+CT-N and CCl₄+AD-N (left) and antibody control (right).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)

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Immunohistochemical analysis of mouse lung. Samples were fixed in 10% buffered formalin, paraffinized and sliced at 1.5 μ m thick. Antigen retrieval was performed using **ab64214** for the deparaffinized slices. Sections were blocked with 2% normal goat serum, PBS(-) and 0.1% Tween20. They were then incubated with the primary antibodies for 1 hour at 4°C and with secondary antibodies for 30 minutes at room temperature. The avidin-biotin-peroxidase complex method with peroxidase streptavidin and the DAB substrate kit ab64238 was performed.

(A) A resected lung from a mouse sacrificed 28 days after SCL injection.

(B) Haematoxylin and Eosin staining showing the tumor composed of a central area with necrosis and a peripheral zone filled with SCLs.

(C) Immunohistochemical staining for MMP-14 showing positive expression of MMP-14 in the peripheral zone of the tumor and negative in central zone.

(D) Immunohistochemical staining weak staining of MMP-2 in the peripheral zone.

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