abcam

Product datasheet

Avidin/Biotin Blocking Kit ab64212

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Overview

Product name Avidin/Biotin Blocking Kit

Product overview Avidin Biotin Blocking Kit ab64212 blocks signal from endogenous avidin, biotin and biotin-binding proteins in tissues when used with biotin-based IHC detection (eg. with ABC IHC detection kits).

When using the kit, firstly an excess of avidin is added to the sample to bind endogenous biotin, that avidin is then blocked with an excess of biotin. Excess biotin and avidin is washed away.

The kit is often used with cells and tissues containing high levels of biotin. This can be indicated by blocking sections with hydrogen peroxide, and then incubating sections with streptavidin-HRP and then DAB; brown DAB staining indicates endogenous biotin. Kidney, liver, spleen especially contain high levels of biotin.

This kit was previously called Endogenous Avidin/Biotin Blocking Kit.

IHC protocol suitable for use with Avidin Biotin Blocking Kit ab64212:
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, block for endogenous biotin by incubating with avidin block for 15 mins, washing twice, incubating with biotin block for 15 mins, and washing twice.**

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 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.

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, ICC/IF, IHC-Fr

Properties

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

Storage buffer Preservative: 0.08% Sodium azide
Constituent: Avidin

Components	15 ml
Avidin Block	1 x 15ml
Biotin Block	1 x 15ml

Relevance Some cells, and tissues such as kidney, liver and spleen, contain endogenous biotin. Using an avidin-biotin staining method may result in high, non-specific background staining. A significant reduction of this non-specific background can be obtained by pre-treatment of cells/tissues with avidin/biotin blocking reagents prior to the incubation of biotinylated antibody.

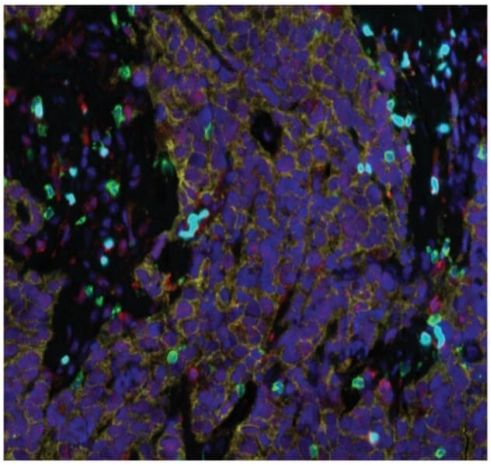
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab64212 in the following tested applications.

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

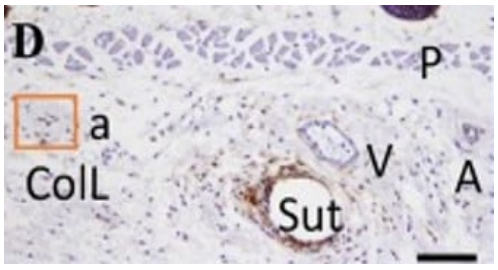
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-Fr		Use at an assay dependent concentration.

Images



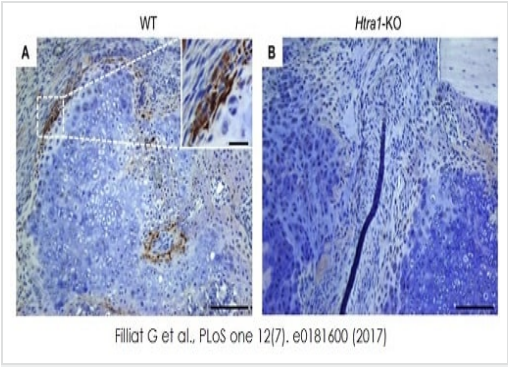
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Avidin/Biotin Blocking Kit (ab64212)

Micrographs showing T-cells infiltrationMulti-spectral, multicolor Immunofluorescence for T-cells infiltration in lymph nodes samples: dMMR tumor. (200x magnification). Multiplex sequential IF staining on 3-?m sections from FFPE tissue; Tissue sections were treated with an avidin/biotin blocking kit (ab64212) before using a biotin conjugated Foxp3 antibody. Other antibodies were conjugated to fluorochroms. CD4:red, CD8: green, CD4/Foxp3: tile, CD8 blue, EPCAM: yellow



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Avidin/Biotin Blocking Kit (ab64212)

Representative histological images of detours at the proximal surgical site. Sut: suture, V: vein, ColL: collecting LV, P: panniculus carnosus muscle, A: arterySpecimens for IHC staining of LVs were washed with PBS comprising 0.03% Tween-20 (PBST), incubated with protein blocking solution, endogenous avidin/biotin blocking kit (ab64212), and 0.03% hydrogen peroxide in methanol. The Syrian Hamster anti-podoplanin/gp36 antibody (ab11936) was used at a dilution of 1:600. Subsequently, the specimens were incubated with secondary antibodies linked with biotin (ab7145) followed by streptavidin linked with HRP (ab7403).



Immunohistochemistry - ab64212

Immunohistochemical analysis staining HTRA1 in mouse bone tissue sections. Tissue sections were dewaxed, rehydrated and treated with Endogenous Avidin/Biotin Blocking Kit (ab64212), 3% H_2O_2 and normal swine serum. Tissue sections were then incubated with a polyclonal anti-HTRA1 antibody for 1 hour at 37°C. After PBS wash, tissue sections are incubated with biotinylated swine anti-rabbit IgG for 45 minutes at 37°C and further incubated for 30 minutes after washing. With Vectastatin. Sections were developed using DAB and counterstained using Harris modified hematoxylin.

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