

Protein Block ab64226

[64 References](#) [6 Images](#)

Overview

Product name	Protein Block
Tested applications	Suitable for: IHC-P, ELISA, WB
General notes	<p>Protein blocking buffer ab64226 for serum-free blocking of non-specific antibody binding in IHC, ELISA and western blot. Ready to use. No mixing or dilution required.</p> <p>For use in ELISA, add Protein Block to well and incubate for 2-10 minutes before addition of sample. Wash and continue procedure. For use in western blotting, incubate for one hour at room temperature or overnight at 4°C.</p>

IHC protocol suitable for use with Protein Block ab64226:

For frozen sections, skip steps 1 and 2. For fluorescent IHC, skip step 3, incubate with fluorescent dye conjugated secondary at step 6, skip rest of steps, and mount with [anti-fade mounting medium](#).

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 ab64226** (or [normal serum](#) from same species as secondary antibody) **and incubate for at least 30 minutes at room temperature** to block nonspecific background staining. Wash once in buffer.
5. Apply primary antibody in [antibody diluent](#) and incubate. Wash 4 times in buffer.
6. Incubate 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. 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](#).

Properties

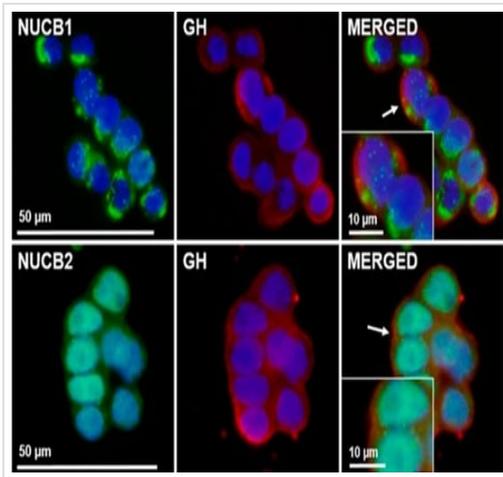
Form	Liquid
Storage instructions	Store at +4°C.
Storage buffer	pH: 7.2 Preservative: 0.1% Sodium azide Constituents: PBS, 0.5% Casein, 0.5% BSA
Reagent notes	ab64226 reduces the handling of animal serums in the laboratory. The need to match species with the secondary antibody is eliminated due to the lack of normal serum in this product. It has been shown to be effective for immunohistochemistry, ELISA, and blot and requires no mixing or diluting.
Relevance	Protein Block is used with immunolabeling techniques for the reduction of non-specific background staining.

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab64226 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.
ELISA		Use at an assay dependent dilution.
WB		Use at an assay dependent dilution.

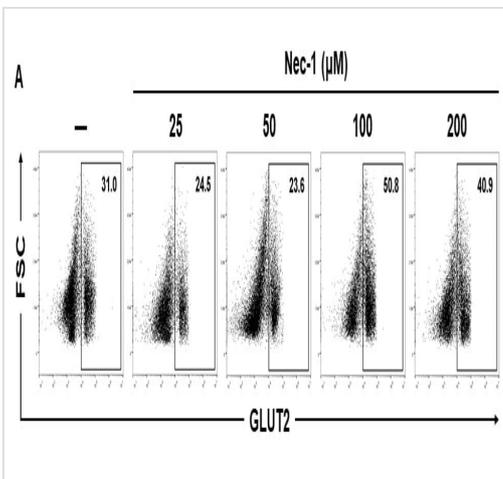
Images



Immunocytochemistry - Protein Block (ab64226)

Image from Velez et al., *Sci Rep.*, 10(1):16686; doi: 10.1038/s41598-020-73840-4. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

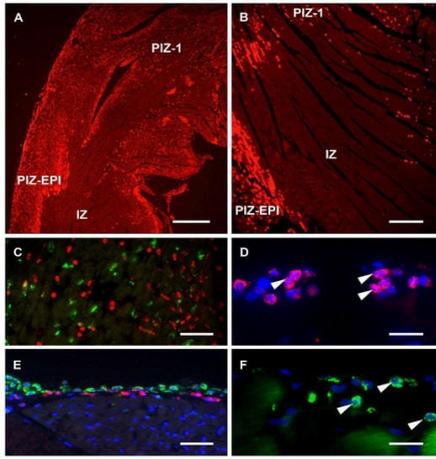
NUCB1/NLP and NUCB2/NESF colocalizes with GH in somatotrophs. Representative images of immunofluorescence detection of NUCB1 (green), NUCB2 (green) and GH (red) in GH3 cells. Cells were blocked with an antibody blocking buffer (ABB) based in PBS consisting of 3% BSA, 0.05% Triton X-100 and 10% of protein block solution (ab64226). Cells were counterstained with DAPI (blue) and the images were acquired at 40X magnification.



Flow Cytometry - Protein Block (ab64226)

Image from Lau, et al., *PLoS ONE* 15, (15):12; e0243506; doi: 10.1371/journal.pone.0243506. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

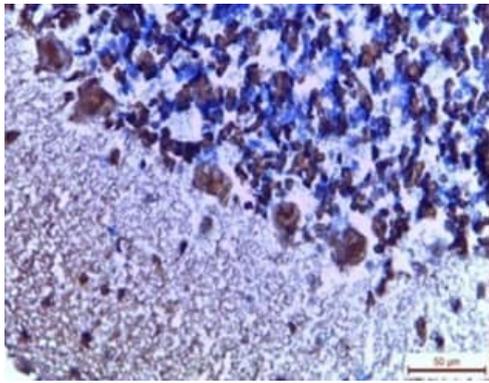
GLUT2 expression in beta cells of islets after treatment with necrostatin-1. Islets were dissociated into single cells, fixed and permeabilised before incubation with Protein Block (ab64226) on ice for 30 minutes to decrease non-specific binding. Then cells were labelled using a FITC-conjugated anti-GLUT2.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Protein Block (ab64226)

Image from Kjucevic et al., *Nutrients*, 11(8):1890; doi: 10.3390/nu11081890. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

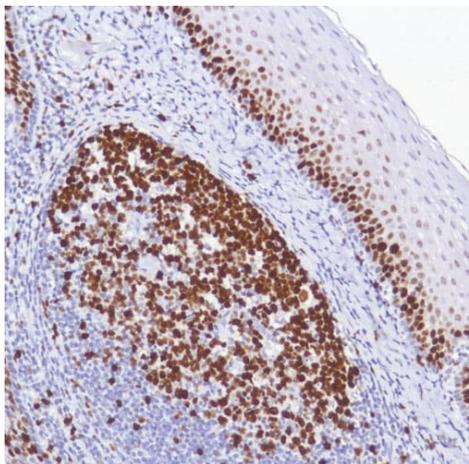
Immunofluorescence staining of MPO and CD68 in rat heart(A,B) MPO (**ab9535**, diluted at 1:100) immunoreactivity on a cross section of the heart showing the transmural ischemic zone (IZ) of the anterior wall of the left ventricle with subepicardial (PIZ-EPI) and peripheral (PIZ-1) peri-infarct zones; (C) MPO- (red, **ab150084**, diluted 1:200) and CD68 (**ab31630**, diluted at 1:250)- immunoreactivity (green, **ab150117**, diluted 1:200) of the peri-infarct zone showing no co-localisation of fluorescence; (D) MPO- immunoreactivity is found in the cytoplasm of polymorphonuclear cells (white arrows); (E) a layer of cellular profiles, immunoreactive for both the MPO and CD68 detected within the subepicardial myocardium; (F) CD68-immunoreactivity is found in the cytoplasm of mononuclear cells (white arrows). Scale bars: A = 0.5 mm, B = 200 μ m, C = 75 μ m, D,F = 20 μ m, E = 50 μ m.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Protein Block (ab64226)

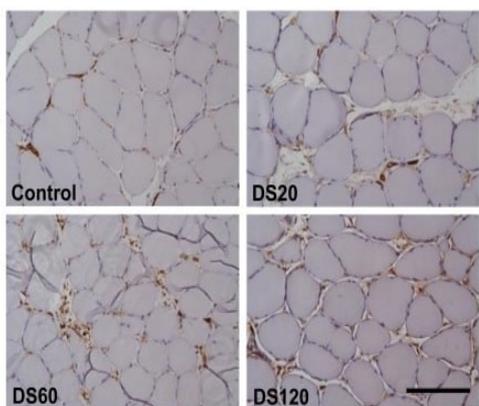
Image from Mbamed et al., *Sci Rep.*, 10: 8840; doi.org/10.1038/s41598-020-64050-z. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Light micrographs of cerebellar sections with immunohistochemistry by anti-caspase 3 antibodies. Adult male albino rat demonstrating mild caspase-3 immunoreactivity in the three cerebellar layers. Incubation of sections was done with rabbit monoclonal caspase-3 antibodies (Abcam, Cambridge, UK), 1:200 dilutions for 1?h.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Protein Block (ab64226)

ab64226 was used as part of the [ab93677](#) anti-Mouse and Rabbit specific HRP (ABC) Detection IHC kit to perform immunohistochemical analysis of Human Tonsil tissue labeling Ki-67.



Yu S.H., PLoS One (9):e114649 (2014)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Protein Block (ab64226)

Yu S.H., PLoS One 9 (12), Fig 7c. doi: 10.1371/journal.pone.0114649 Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

ab64226 was used in the mouse and rabbit specific HRP/DAB detection IHC Kit ([ab64264](#)) to visualize immunohistochemical analysis staining CD163 in mouse soleus muscle section. Samples were incubated in rabbit CD163 antibody at 1:450 dilution.

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