abcam

Product datasheet

Anti-6X His tag® antibody [EPR20547] - BSA and Azide free ab232492

Recombinant RabMAb

7 Images

Overview

Product name	Anti-6X His tag® antibody [EPR20547] - BSA and Azide free
Description	Rabbit monoclonal [EPR20547] to 6X His tag® - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ChIP, IHC-P, WB, Flow Cyt, ICC/IF, IP
Species reactivity	**Reacts with:** Species independent
Immunogen	Synthetic peptide corresponding to 6X His tag®.
Positive control	IHC-P: Agarose embedded HEK-293T cells.
General notes	Ab232492 is the carrier-free version of ab213204. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab232492 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20547
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab232492** in the following tested applications.

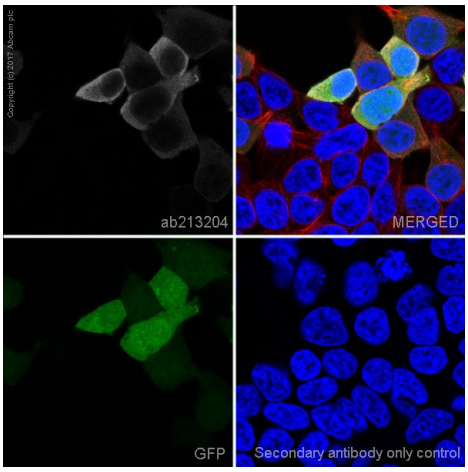
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. We don't recommend this antibody for mouse in IHC. In our hands mouse tissues showed non-specific staining.
WB		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Relevance	The H-H-H-H-H-H motif is used as a tag on many recombinant proteins to facilitate purification. The antibody recognizes the His-tag fused to the amino- or carboxy- termini of targeted proteins in transfected or transformed cells.
Cellular localization	Depends upon the localization of the parent protein tagged with hexahistidine.

Images



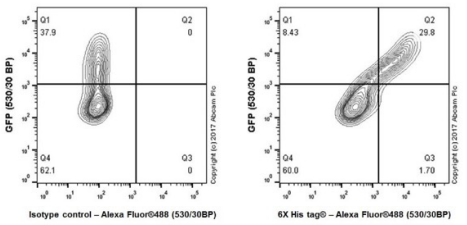
Immunocytochemistry/ Immunofluorescence - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with GFP-Myc-His vector expression construct labeling 6X His tag® with ab213204 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (white). Confocal image showing positive staining on HEK-293T cells transfected with GFP-Myc-His vector expression construct.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab213204).

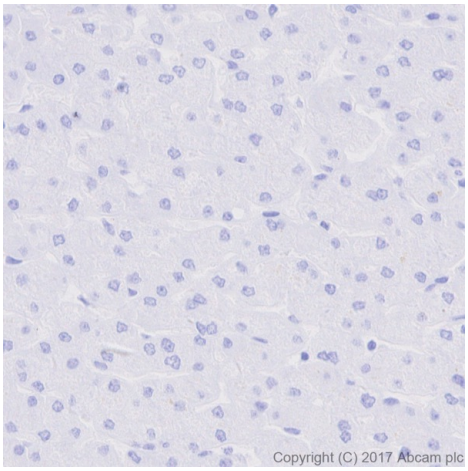


Flow Cytometry - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with GFP-Myc-His vector labeling 6X His tag® with ab213204 (right panel) at 1/5000 dilution compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (left panel). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150079) at 1/2000 dilution was used as the secondary antibody.

Gate is set between transfected and untransfected HEK-293T cells.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab213204).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

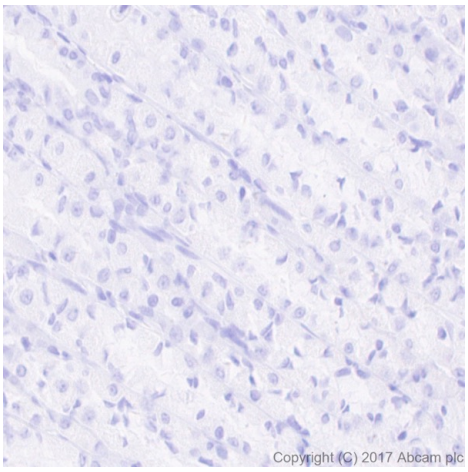
Immunohistochemical analysis of paraffin-embedded human liver tissue labeling 6X His tag® with ab213204 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Negative control: No staining on human liver.

Counter stained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab213204).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

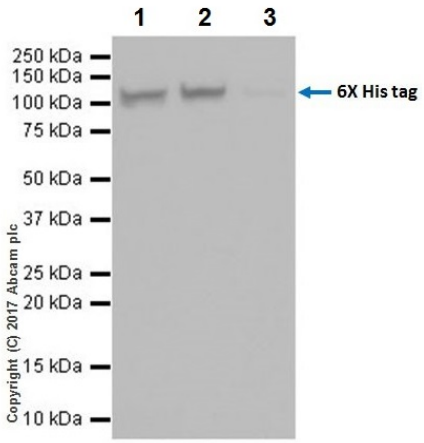
Immunohistochemical analysis of paraffin-embedded rat stomach tissue labeling 6X His tag® with ab213204 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Negative control: No staining on rat stomach.

Counter stained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab213204).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

His-tagged Staphylococcus aureus cas9 was immunoprecipitated from 0.35 mg of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with a His-tagged Staphylococcus aureus cas9 (J7RUA5; aa1-1053; 125 kDa) construct, whole cell lysate with ab213204 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab213204 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: HEK-293T transfected with His-tagged Staphylococcus aureus cas9 construct, whole cell lysate 10 µg (Input).

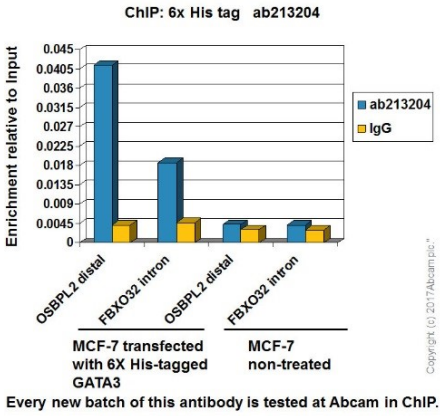
Lane 2: ab213204 IP in HEK-293T transfected with His-tagged Staphylococcus aureus cas9 construct, whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab213204 in HEK-293T transfected with His-tagged Staphylococcus aureus cas9 construct, whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab213204).

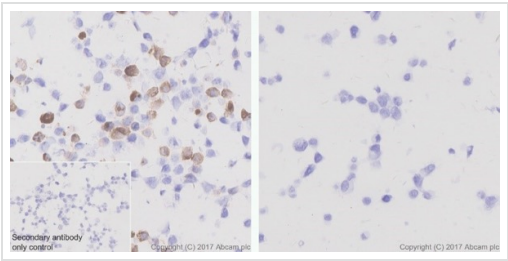


ChIP - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

Chromatin was prepared from MCF7 (human breast adenocarcinoma cell line) cells transfected with 6X His-tagged GATA3 according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab213204 (blue), and 20µl of A/G sepharose beads slurry (10µl of sepharose A beads + 10µl of sepharose G beads). 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

ChIP was performed according to the literature (PMID:22951069).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab213204).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

Immunohistochemical analysis of agarose-embedded HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with a His-tagged Staphylococcus aureus cas9 (J7RUA5; aa1-1053; 125kDa) construct labeling 6X His tag® with ab213204 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Left image: Positive staining on HEK-293T transfected with a His-tagged Staphylococcus aureus cas9 (J7RUA5; aa1-1053; 125kDa) construct. **Right image:** No staining on HEK-293T transfected with an empty expression vector.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab213204).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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