abcam

ab232492

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

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20547
Purification technique	Affinity purification Protein A
Concentration	0.946 - 1.042 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Tag
Dilution info	-

Notes	-

IP

Tested

Species	Tag
Dilution info	-
Notes	-

ChIP

Tested

Species	Tag
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Tag
Dilution info	-
Notes	-

WB

Tested

Species	Tag
Dilution info	-
Notes	-

IHC-P

Tested

Species	Tag
Dilution info	-
Notes	We don't recommend this antibody for mouse in IHC. In our hands mouse tissues showed non-specific staining. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information 6X His tag®

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab232492 is the carrier-free version of ab213204.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

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.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The 6X His tag is a sequence commonly used for protein purification detection and immobilization. It consists of six histidine residues that bind to metal ions like nickel or cobalt. This makes it a highly efficient tag for affinity chromatography. His tag antibodies such as anti-His HRP facilitate the detection of His-tagged proteins in various assays. Being a small tag with a typical molecular weight of about 0.84 kDa it minimally interferes with protein function. Many proteins expressed in E. coli and other expression systems carry the 6X His tag for ease of purification and analysis.
Biological function summary	Proteins tagged with the 6X His tag often play roles in studies involving recombinant proteins. The tag itself does not alter the biological function of the target protein. It is not part of any natural protein complex but aids in the study of such complexes. Researchers use His tag antibodies like anti-His to specifically target and study these tagged proteins without cross-reactivity to other proteins.
Pathways	The incorporation of the 6X His tag into proteins allows them to be studied more efficiently in various biological pathways. It can be part of pathways like signal transduction or metabolic pathways. When tagged proteins interact with other molecules such as kinases or receptors researchers can track these interactions using the tag and anti-Histidine antibodies. This technique informs on proteins that are hard to purify due to low abundance or instability.
Associated diseases and disorders	The 6X His tag itself does not have direct implications. However proteins of interest in disease models often carry the tag which allows researchers to purify and study them in detail. For example cancer-related proteins may be expressed with a His tag to understand their role in tumorigenesis. Similarly proteins involved in metabolic disorders could be tagged which helps in analyzing specific interactions or functions disrupted in diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

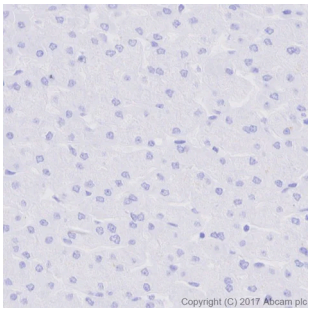
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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9 product images



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

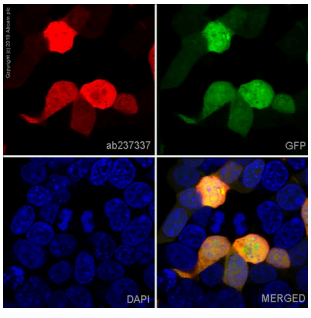
6X His tag® Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-6X His tag® antibody

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).

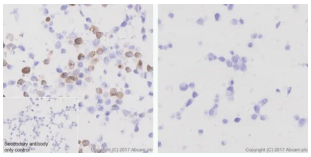
Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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

6X His tag® Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-6X His tag® antibody

Clone EPR20547 (ab232492) has been successfully conjugated by Abcam. This image was generated using Anti-6X His tag® antibody [EPR20547] (Alexa Fluor® 647). Please refer to ab237337 for protocol details.
ab237337 staining 6X His tag® in 293T cells transfected with 6X His tag® with GFP tag. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab237337 at 1/100 dilution (shown in red). Nuclear DNA was labeled with DAPI (shown in blue) and GFP is shown in green.



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

6X His tag® Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-6X His tag® antibody

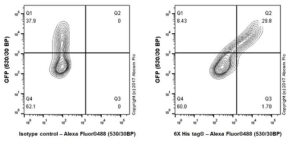
Immunohistochemical analysis of agarose-embedded HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with a His-tagged Staphylococcus aureus cas9 (J7RUA5; aa1-1053; 125kDa) construct labeling 6X His tag® with ab213204 at 1/16000 dilution, followed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

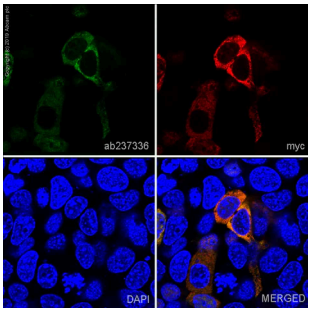


Flow Cytometry - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

6X His tag® Flow Cytometry staining using rabbit Anti-6X His tag® antibody

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).

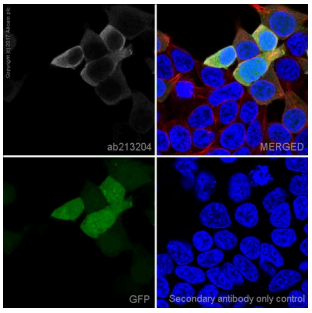


Immunocytochemistry/ Immunofluorescence - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

6X His tag® Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-6X His tag® antibody

Clone EPR20547 (ab232492) has been successfully conjugated by Abcam. This image was generated using Anti-6X His tag® antibody [EPR20547] (Alexa Fluor® 488). Please refer to ab237336 for protocol details.

ab237336 staining 6X His tag® in 293T cells transfected with 6X His tag® with MYC tag. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab237336 at 1/200 dilution (shown in green) and Mouse monoclonal to Myc-Tag (Alexa Fluor® 647) (shown in red). Nuclear DNA was labeled with DAPI (shown in blue).



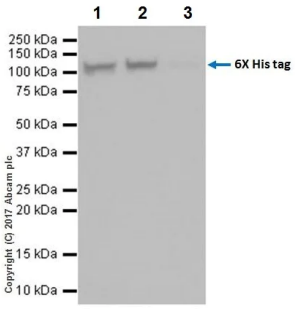
Immunocytochemistry/ Immunofluorescence - Anti-6X His tag® antibody [EPR20547] - BSA and Azide free (ab232492)

6X His tag® Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-6X His tag® antibody

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).



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.

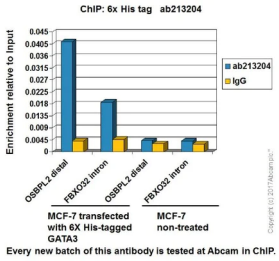
Blocking/Dilution buffer: 5% NFDM/TBST.

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).

All lanes:
Immunoprecipitation - Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204)

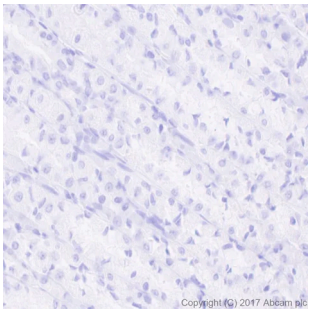
Observed band size: 125 kDa



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)

6X His tag® Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-6X His tag® antibody

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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.