

Product datasheet

Anti-6X His tag® antibody [EPR20547] - ChIP Grade ab213204

Recombinant **RabMAB**

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Overview

Product name	Anti-6X His tag® antibody [EPR20547] - ChIP Grade
Description	Rabbit monoclonal [EPR20547] to 6X His tag® - ChIP Grade
Host species	Rabbit
Tested applications	Suitable for: WB, ICC/IF, IP, Flow Cyt, ChIP, IHC-P
Immunogen	Synthetic peptide corresponding to 6X His tag®.
Positive control	WB: His-tagged mouse Cathepsin D and CDKN2A/p16INK4a proteins; HEK-293T transfected with His-tagged Staphylococcus aureus cas9, human Brachyury, human PRDM9 and human GAPDH cell lysates. IHC-P: HEK-293T cells transfected with His-tagged S.aureus cas9. ICC/IF: HEK-293T cells transfected with GFP-Myc-His. Flow Cyt: HEK-293T cells transfected with GFP-Myc-His. IP: HEK-293T cells transfected with His-tagged S.aureus cas9 cell lysate. ChIP: Chromatin from MCF7 cells transfected with 6X His-tagged GATA3.
General notes	<p>Our RabMAB® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents</p> <p>This product is a recombinant rabbit monoclonal antibody.</p>

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	Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20547
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab213204** 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
WB		1/5000.
ICC/IF		1/100.
IP		1/30.
Flow Cyt		1/5000.
ChIP		Use 2 µg for 25 µg of chromatin.
IHC-P		1/16000. 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.

Target

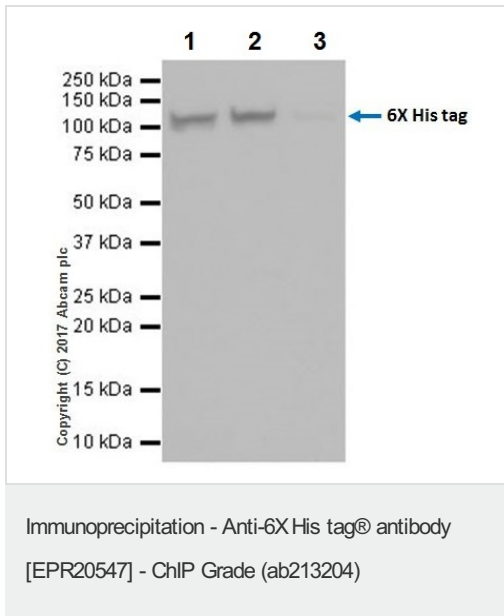
Relevance

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



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 secondary antibody (HRP) (ab131366), was used as secondary antibody at 1/10000 dilution.

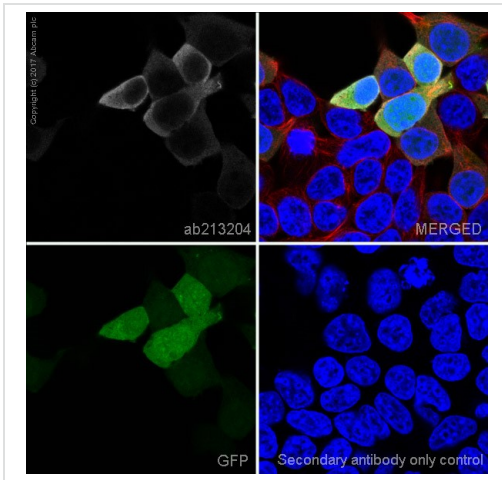
Lane 1: HEK-293T transfected with His-tagged *Staphylococcus aureus* cas9 construct, whole cell lysate 10 µg (Input).

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.

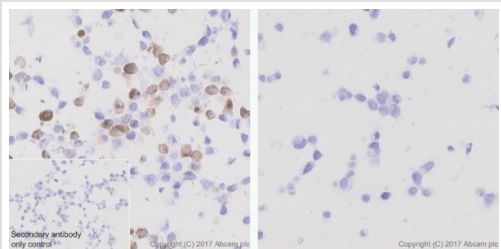


Immunocytochemistry/ Immunofluorescence - Anti-6X His tag[®] antibody [EPR20547] - ChIP Grade (ab213204)

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.



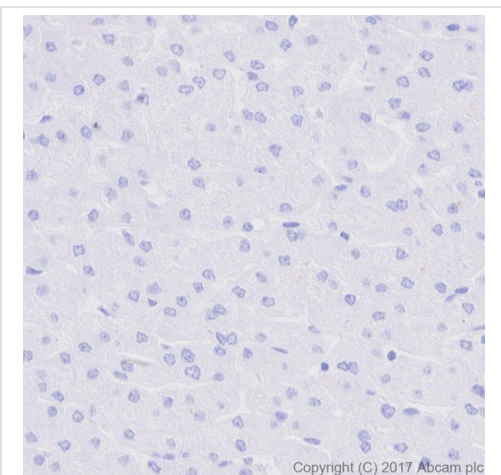
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204)

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.

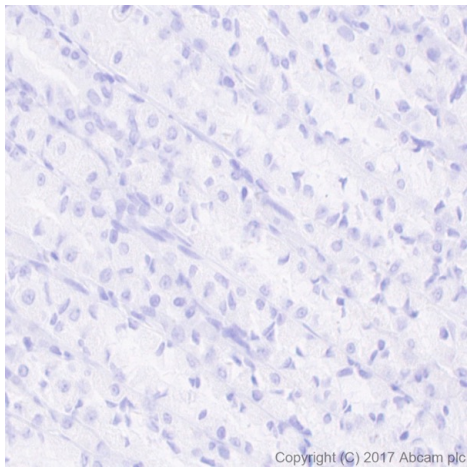


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204)

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.



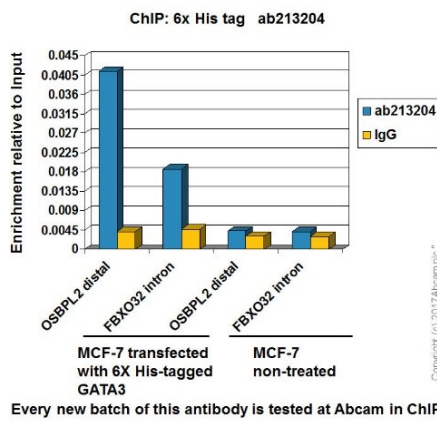
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204)

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.

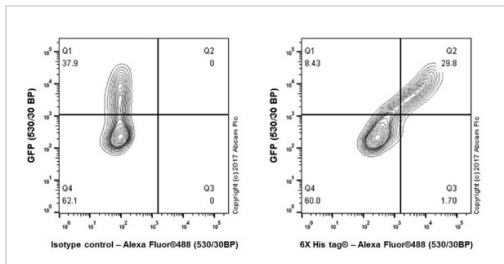
Counter stained with Hematoxylin.



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

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).

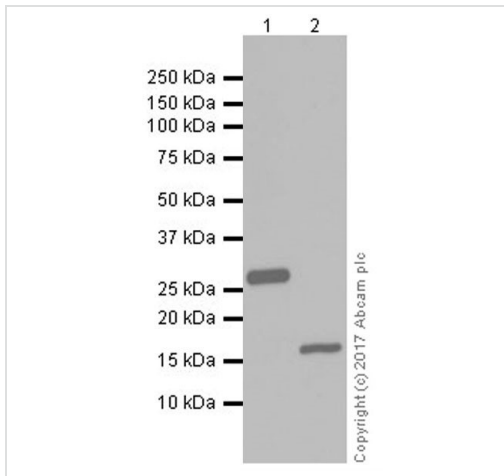
ChIP was performed according to the literature (PMID:22951069).



Flow Cytometry - Anti-6X His tag® antibody
[EPR20547] - ChIP Grade (ab213204)

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.



Western blot - Anti-6X His tag® antibody
[EPR20547] - ChIP Grade (ab213204)

All lanes : Anti-6X His tag® antibody
[EPR20547] - ChIP Grade (ab213204) at
1/20000 dilution

Lane 1 : His-tagged mouse Cathepsin D
recombinant protein (aa65-310; 27kDa)

Lane 2 : His-tagged mouse
CDKN2A/p16INK4a recombinant protein
(aa1-168;18kDa)

Lysates/proteins at 0.02 µg per lane.

Secondary

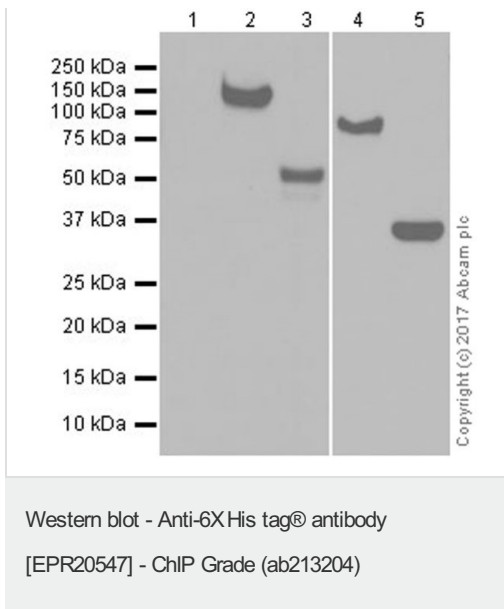
All lanes : Goat Anti-Rabbit IgG H&L (HRP)
(ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 18,27 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDm/TBST.



All lanes : Anti-6X His tag® antibody
[EPR20547] - ChIP Grade (ab213204) at
1/5000 dilution

Lane 1 : HEK-293T (human epithelial cell from embryonic kidney transformed with large T antigen) transfected with an empty expression vector, whole cell lysate

Lane 2 : HEK-293T (human epithelial cell from embryonic kidney transformed with large T antigen) transfected with a His-tagged Staphylococcus aureus cas9 (J7RUA5; aa1-1053;125kDa) construct, whole cell lysate

Lane 3 : HEK-293T (human epithelial cell from embryonic kidney transformed with large T antigen) transfected with a His-tagged human Brachyury (O15178; aa1-435;50kDa) construct, whole cell lysate

Lane 4 : HEK-293T (human epithelial cell from embryonic kidney transformed with large T antigen) transfected with a His-tagged human PRDM9 (Q9NQV7; aa1-894;100kDa) construct, whole cell lysate

Lane 5 : HEK-293T (human epithelial cell from embryonic kidney transformed with large T antigen) transfected with a His-tagged human GAPDH (P04406; aa1- 335;36kDa) construct, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 100,125,36,50 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time: Lane 1-3: 1 second; Lane 4-5: 10 seconds.

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