abcam

Product datasheet

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

יעלאעבע RabMAb

★★★★ 1 Abreviews 14 References 画像数 10

製品の概要

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

製品の詳細 Rabbit monoclonal [EPR20547] to 6X His tag® - ChIP Grade

Rabbit 由来種

アプリケーション 適用あり: WB, ICC/IF, IP, Flow Cyt, ChIP, IHC-P

種交差性 交差種: Recombinant fragment

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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**.

製品の特性

製品の状態

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol, PBS

精製度 Protein A purified

ポリたノ モノクローナル **PR2**0547

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab213204の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

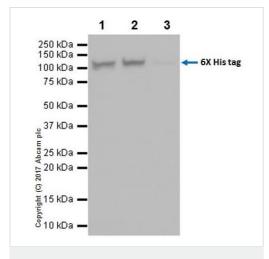
関連性 The H-H-H-H motif is used as a tag on many recombinant proteins to facilitate purification.

His-tags can be fused to the amino- or carboxy- termini of proteins in transfected or transformed

cells.

細胞内局在 Depends upon the localization of the parent protein tagged with hexahistidine.

画像



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

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 1/10,000 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 (<u>ab172730</u>) 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.

ab213204

MERGED

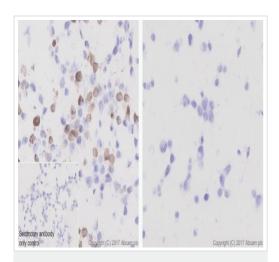
Secondary antibody only control

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 lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-6X His tag® antibody

[EPR20547] - ChIP Grade (ab213204)

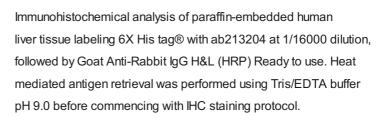
Immunohistochemical analysis of agarose-embedded HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with a His-tagged Staphylococcus aureus cas9 (J7RUA5; aa1-1053; 125kDa) construct labeling 6X His tag® with ab213204 at 1/16000 dilution, followed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

Left image: Positive staining on HEK-293T transfected with a Histagged 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.

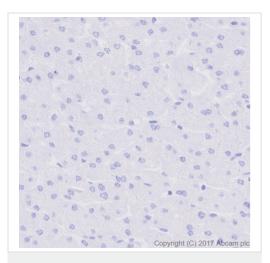
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

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



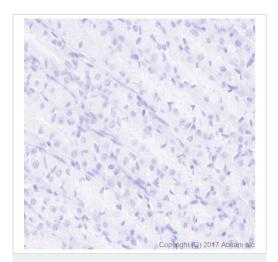
Negative control: No staining on human liver.

Counter stained with Hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-6X His tag® antibody

[EPR20547] - ChIP Grade (ab213204)

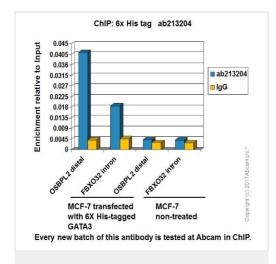


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-6X His tag® antibody
[EPR20547] - ChIP Grade (ab213204)

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

Negative control: No staining on rat stomach.

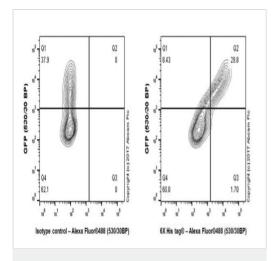
Counter stained with Hematoxylin.



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 25 μ g of chromatin, 2 μ g of ab213204 (blue), and 20 μ l of A/G sepharose beads slurry (10 μ l of sepharose A beads + 10 μ l of sepharose G beads). 2 μ g of rabbit normal lgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

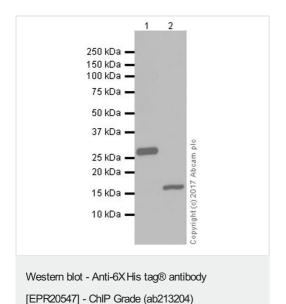
ChIP was performed according to the literature (PMID:22951069).



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 lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (left panel). Goat Anti-Rabbit lgG 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.



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/p16lNK4a recombinant protein (aa1-168;18kDa)

Lysates/proteins at 0.02 µg per lane.

Secondary

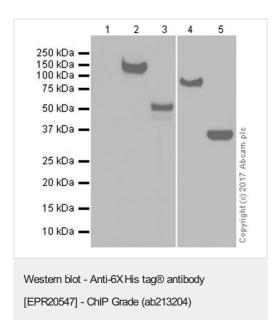
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

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

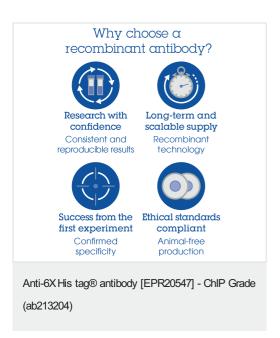
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

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.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- · Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.co.jp/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors