abcam

Product datasheet

Anti-KAP1 antibody [EPR5249] - BSA and Azide free ab247904

KO 評価済 リコンビナント RabMAb

画像数 8

製品の概要

製品名	Anti-KAP1 antibody [EPR5249] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EPR5249] to KAP1 - BSA and Azide free
由来種	Rabbit
特異性	Although the immunogen is from a phosphor-peptide, this antibody detects phospho and non-phospho KAP1. Based on a peptide blocking experiment it has been found that the signal generated after non-phospho peptide blocking became much weaker, thus indicating that this antibody shows cross-reactivity with the non-phospho KAP1 at high level.
アプリケーション	**適用あり:** IP, ICC/IF, WB, IHC-P, Flow Cyt (Intra)
種交差性	**交差種:** Human
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: A431 and MCF7 cell lysates; IHC-P: Human colon and kidney tissue; ICC/IF: Hela cells; IP: A431 cell lysate. FC (Intra): HeLa cells.
特記事項	ab247904 is the carrier-free version of **ab109545**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.2 Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR5249
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab247904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。

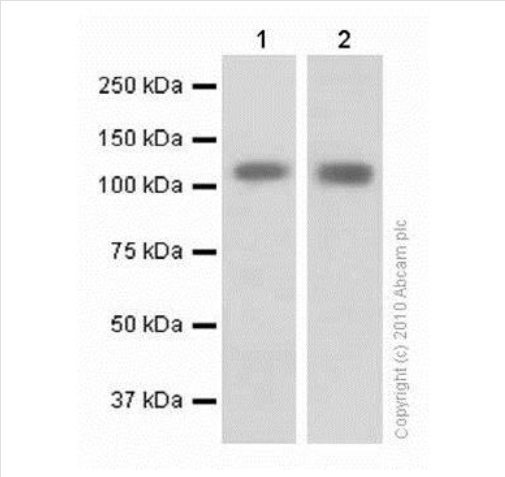
アプリケーション	Abreviews	特記事項
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 89 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. For antigen retrieval, heat up to 98 degree C, below boiling, and then let cool for 10-20 minutes.
Flow Cyt (Intra)		Use at an assay dependent concentration.

ターゲット情報

機能 Nuclear corepressor for KRAB domain-containing zinc finger proteins (KRAB-ZFPs). Mediates gene silencing by recruiting CHD3, a subunit of the nucleosome remodeling and deacetylation (NuRD) complex, and SETDB1 (which specifically methylates histone H3 at 'Lys-9' (H3K9me)) to the promoter regions of KRAB target genes. Enhances transcriptional repression by coordinating the increase in H3K9me, the decrease in histone H3 'Lys-9 and 'Lys-14' acetylation (H3K9ac and H3K14ac, respectively) and the disposition of HP1 proteins to silence gene expression.

	Recruitment of SETDB1 induces heterochromatinization. May play a role as a coactivator for CEBPB and NR3C1 in the transcriptional activation of ORM1. Also corepressor for ERBB4. Inhibits E2F1 activity by stimulating E2F1-HDAC1 complex formation and inhibiting E2F1 acetylation. May serve as a partial backup to prevent E2F1-mediated apoptosis in the absence of RB1. Important regulator of CDKN1A/p21(CIP1). Has E3 SUMO-protein ligase activity toward itself via its PHD-type zinc finger.
組織特異性	Expressed in all tissues tested including spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes.
パスウェイ	Protein modification; protein sumoylation.
配列類似性	Belongs to the TRIM/RBCC family. Contains 2 B box-type zinc fingers. Contains 1 bromo domain. Contains 1 PHD-type zinc finger. Contains 1 RING-type zinc finger.
ドメイン	The HP1 box is both necessary and sufficient for HP1 binding. The PHD-type zinc finger enhances CEBPB transcriptional activity. The PHD-type zinc finger, the HP1 box and the bromo domain, function together to assemble the machinery required for repression of KRAB domain-containing proteins. Acts as an intramolecular SUMO E3 ligase for autosumoylation of bromodomain. The RING-finger-B Box-coiled-coil/tripartite motif (RBCC/TRIM motif) is required for interaction with the KRAB domain of KRAB-zinc finger proteins. Binds four zinc ions per molecule. The RING finger and the N-terminal of the leucine zipper alpha helical coiled-coil region of RBCC are required for oligomerization. Contains one Pro-Xaa-Val-Xaa-Leu (PxVxL) motif, which is required for interaction with chromoshadow domains. This motif requires additional residues -7, -6, +4 and +5 of the central Val which contact the chromoshadow domain.
翻訳後修飾	Phosphorylated upon DNA damage, probably by ATM or ATR. ATM-induced phosphorylation on Ser-824 represses sumoylation leading to the de-repression of expression of a subset of genes involved in cell cycle control and apoptosis in response to genotoxic stress. Dephosphorylation by the phosphatases, PPP1CA and PP1CB forms, allows sumoylation and expression of TRIM28 target genes. Sumoylation/desumoylation events regulate TRIM28-mediated transcriptional repression. Sumoylation is required for interaction with CHD3 and SETDB1 and the corepressor activity. Represses and is repressed by Ser-824 phosphorylation. Enhances the TRIM28 corepressor activity, inhibiting transcriptional activity of a number of genes including GADD45A and CDKN1A/p21. Lys-554, Lys-779 and Lys-804 are the major sites of sumoylation. In response to Dox-induced DNA damage, enhanced phosphorylation on Ser-824 prevents sumoylation and allows de-repression of CDKN1A/p21.
細胞内局在	Nucleus. Associated with centromeric heterochromatin during cell differentiation through CBX1.

画像



Western blot - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

All lanes : Anti-KAP1 antibody [EPR5249] (**ab109545**) at 1/10000 dilution

Lane 1 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

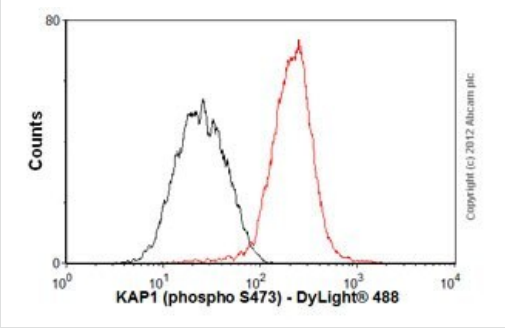
Developed using the ECL technique.

Predicted band size: 89 kDa

Exposure time: 10 seconds

This data was developed using **ab109545**, the same antibody clone in a different buffer formulation:

Blocking/diluting buffer and concentration: 5% NFDM/TBST

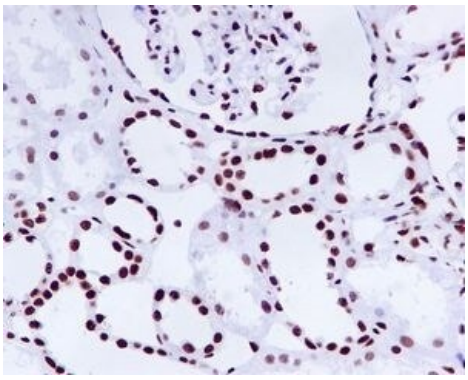


Flow Cytometry (Intracellular) - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

This data was developed using **ab109545**, the same antibody clone in a different buffer formulation:

Overlay histogram showing HeLa cells stained with **ab109545** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab190545**, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of

>5,000 events was performed.

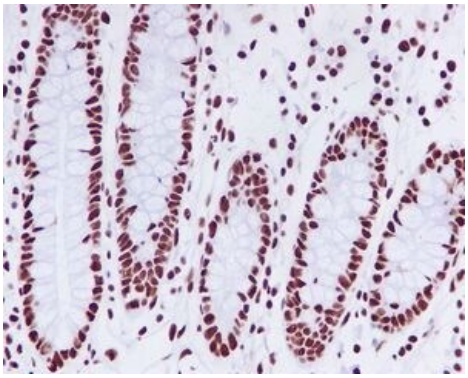


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

This data was developed using **ab109545**, the same antibody clone in a different buffer formulation:

ab109545, at a 1/100 dilution, staining KAP1 in formalin-fixed, paraffin-embedded Human kidney tissue.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

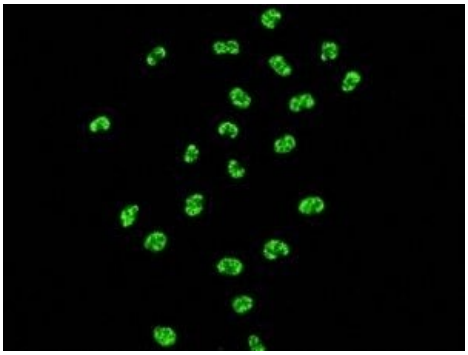


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

This data was developed using **ab109545**, the same antibody clone in a different buffer formulation:

ab109545, at a 1/100 dilution, staining KAP1 in formalin-fixed, paraffin-embedded Human colon tissue.

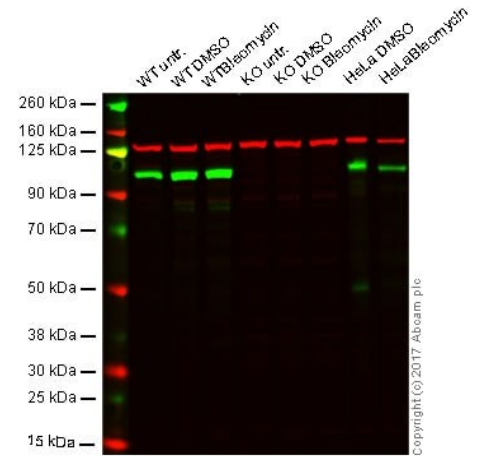
Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

This data was developed using **ab109545**, the same antibody clone in a different buffer formulation:

ab109545, at a 1/100 dilution, staining KAP1 in HeLa cells



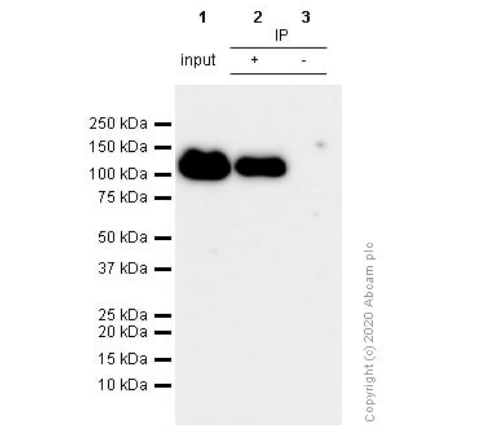
Western blot - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

This data was developed using **ab109545**, the same antibody clone in a different buffer formulation:

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: HAP1 + DMSO whole cell lysate (20 µg)
Lane 3: HAP1 + Blaomycin whole cell lysate (20 µg)
Lane 4: TRIM28 knockout HAP1 whole cell lysate (20 µg)
Lane 5: TRIM28 knockout HAP1 + DMSO whole cell lysate (20 µg)
Lane 6: TRIM28 knockout HAP1 + Blaomycin whole cell lysate (20 µg)
Lane 7: HeLa + DMSO whole cell lysate (20 µg)
Lane 8: HeLa + Blaomycin whole cell lysate (20 µg)

Lanes 1 - 8: Merged signal (red and green). Green - **ab109545** observed at 110 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab109545 was shown to specifically react with KAP1 in wild type cells as signal was lost in KAP1 knockout cells. Wild-type and KAP1 knockout samples were subjected to SDS-PAGE. **ab109545** and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 was immunoprecipitated from 0.35 mg A431 (Human epidermoid carcinoma epithelial cell) cell lysate 10 µg with **ab109545** at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab109545** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: A431 (Human epidermoid carcinoma epithelial cell) cell lysate 10 µg

Lane 2: **ab109545** IP in A431 cell lysate

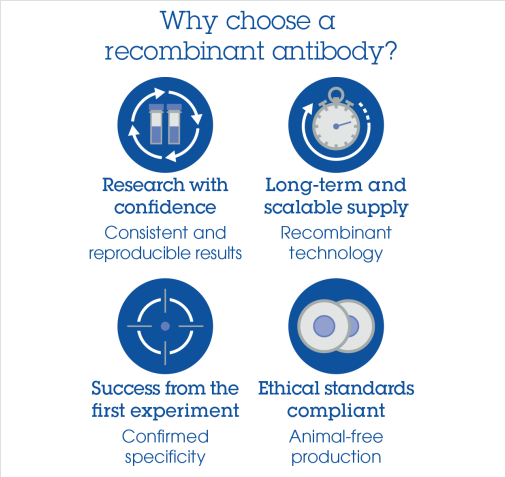
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab109545** in A431 cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 32 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (**ab109545**).



Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

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