abcam

Product datasheet

# Anti-KAP1 antibody [20C1] ab22553

14 Abreviews 78 References 画像数 4

## 製品の概要

| | |
|---|---|
| **製品名** | Anti-KAP1 antibody [20C1] |
| **製品の詳細** | Mouse monoclonal [20C1] to KAP1 |
| **由来種** | Mouse |
| **アプリケーション** | **適用あり:** WB, ICC, IHC-P |
| **種交差性** | **交差種:** Human |
| **免疫原** | Recombinant fragment, corresponding to amino acids 60-383 of Human KAP1. |
| **ポジティブ・コントロール** | IHC-P: Human spleen tissue. WB: HEK-293 (untreated and treated with hydrogen peroxide at 100 uM for 10 min), A549, HEL 92.1.7, PC-3, Caco-2 and BJ fibroblast cell lysates. ICC: HeLa cells. |
| **特記事項** | The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.<br>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As |

## 製品の特性

| | |
|---|---|
| **製品の状態** | Liquid |
| **保存方法** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. |
| **バッファー** | Preservative: 0.05% Sodium azide<br>Constituents: PBS, 0.1% BSA |
| **精製度** | Protein G purified |
| **ポリ/モノ** | モノクローナル |
| **クローン名** | 20C1 |
| **アイソタイプ** | IgG1 |

## 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ab22553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** | ★★★★★ (7) | 1/500. Predicted molecular weight: 88 kDa. |
| **ICC** | | Use a concentration of 2 µg/ml. |
| **IHC-P** | | Use a concentration of 5 µg/ml. |

## 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.

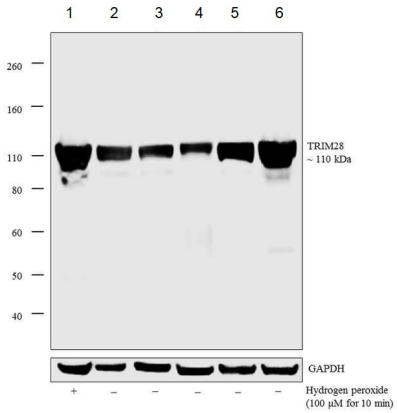
**翻訳後修飾**

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 [20C1] (ab22553)

**All lanes :** Anti-KAP1 antibody [20C1] (ab22553) at 1 µg/ml

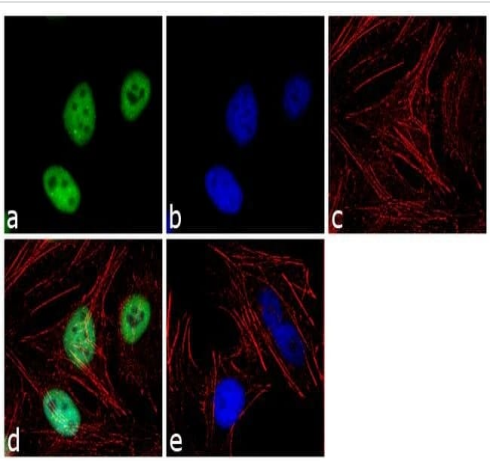
**Lanes 1-2 :** HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
**Lane 3 :** A549 (Human lung carcinoma cell line) whole cell lysate
**Lane 4 :** HEL 92.1.7 whole cell lysate
**Lane 5 :** PC-3 (Human prostate adenocarcinoma cell line) whole cell lysate
**Lane 6 :** Caco-2 (Human colorectal adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 30 µg per lane.

**Predicted band size:** 88 kDa
**Observed band size:** 110 kDa

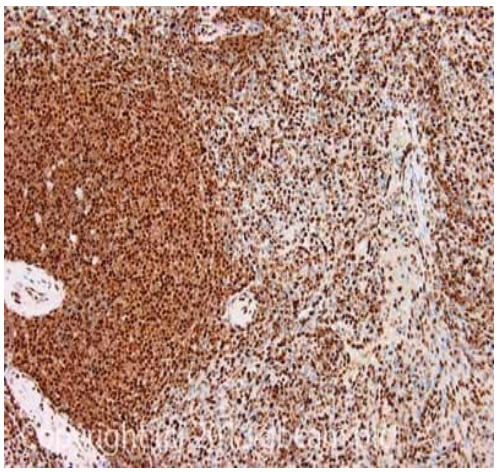
Western blot analysis was performed on nuclear enriched extracts (30 µg lysate) of cells treated with Hydrogen peroxide (100 µm Hydrogen peroxide for 10 min) or untreated. The blots were probed with ab22553 at 1 µg/mL and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate. A 110 kDa band corresponding to KAP1 was observed across the cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel, XCell SureLock™ Electrophoresis System and Novex® Sharp Pre-Stained Protein Standard. Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System. The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using

Pierce™ ECL Western Blotting Substrate.




Immunocytochemistry - Anti-KAP1 antibody [20C1] (ab22553)

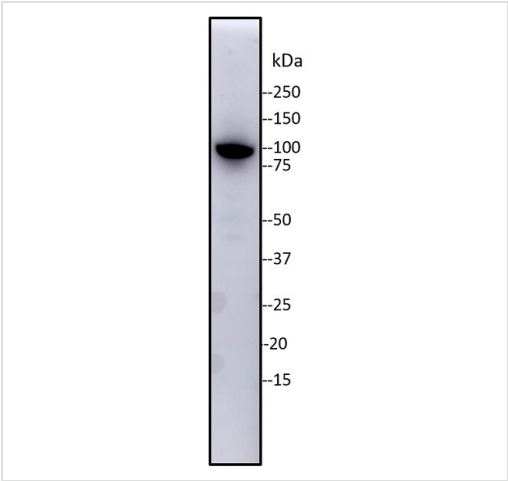
Immunofluorescence analysis of TIF1 beta was performed using 70% confluent log phase HeLa (Human cervix adenocarcinoma epithelial cell) cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with ab22553 at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel c: red) was stained with Rhodamine Phalloidin. Panel d represents the merged image showing nuclear localization. Panel e shows the no primary antibody control. The images were captured at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 antibody [20C1] - ChIP Grade (ab22553)

IHC image of KAP1 staining in Human normal spleen formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab22553, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.




Western blot - Anti-KAP1 antibody [20C1] (ab22553)

Anti-KAP1 antibody [20C1] (ab22553) at 1/500 dilution + Whole cell human BJ fibroblast protein lysate at 10 µg

**Predicted band size:** 88 kDa

Western blot analysis of TRIM28 was performed by loading 10 g of whole cell human BJ fibroblast protein lysate and run on a 4-12% BTE gel. Proteins were transferred to PVDF membrane. Membrane was blocked in 5% non-fat milk in TBST. TRIM28 was detected at approximately 90 kDa using ab22553 at a dilution of 1:500 (2 µg/mL) in 5% milk, followed by a 1:10,000 dilution of anti-mouse HRP. Chemiluminescent detection was performed using SuperSignal West Pico PLUS substrate.



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