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# Anti-KAP1/TRIM28 Antibody Picoband™ (monoclonal, 3H2)

Catalog # ABO14933

## Product Information

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| **Application** | WB, IHC, IHC-F, IF, ICC, FC |
| **Primary Accession** | Q13263 |
| **Host** | Mouse |
| **Isotype** | Mouse IgG2b |
| **Reactivity** | Rat, Human, Mouse |
| **Clonality** | Monoclonal |
| **Format** | Lyophilized |
| **Description** | Anti-KAP1/TRIM28 Antibody Picoband™ (monoclonal, 3H2) . Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. |
| **Reconstitution** | Add 0.2ml of distilled water will yield a concentration of 500ug/ml. |

## Additional Information

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| **Gene ID** | 10155 |
| **Other Names** | Transcription intermediary factor 1-beta, TIF1-beta, E3 SUMO-protein ligase TRIM28, 2.3.2.27, KRAB-associated protein 1, KAP-1, KRAB-interacting protein 1, KRIP-1, Nuclear corepressor KAP-1, RING finger protein 96, RING-type E3 ubiquitin transferase TIF1-beta, Tripartite motif-containing protein 28, TRIM28 (HGNC:16384), KAP1, RNF96, TIF1B |
| **Calculated MW** | 88550 |
| **Application Details** | Western blot, 0.1-0.5 □g/ml, Human, Mouse, Rat<br>Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human, Mouse, Rat<br>Immunohistochemistry (Frozen Section), 0.5-1 □g/ml, Human, Rat<br>Immunocytochemistry/Immunofluorescence, 2 □g/ml, Human<br>Immunofluorescence, 2 □g/ml, Human<br>Flow Cytometry, 1-3 □g/1x10^6 cells, Human |
| **Subcellular Localization** | Nucleus. |
| **Tissue Specificity** | Expressed in all tissues tested including spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes. |
| **Source** | Eukaryota |
| **Contents** | Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg $Na_2HPO_4$, 0.05mg $NaN_3$. |

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| **Clone Names** | Clone: 3H2 |
| **Immunogen** | E.coli-derived human KAP1 recombinant protein (Position: A699-P835). Human KAP1 shares 94.9% amino acid (aa) sequence identity with both mouse and rat KAP1. |
| **Purification** | Immunogen affinity purified. |
| **Cross Reactivity** | No cross-reactivity with other proteins. |
| **Storage** | Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles. |

## Protein Information

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| **Name** | TRIM28 ( HGNC:16384) |
| **Synonyms** | KAP1, RNF96, TIF1B |
| **Function** | E3 SUMO and ubiquitin ligase that plays a pivotal role in embryonic development, genomic imprinting, and maintenance of genomic stability through repression of repetitive and retroviral elements. Also involved in DNA repair, regulation of innate immunity or cellular energy homeostasis. Acts as a scaffold for assembling transcriptional repression complexes containing methyltransferases, histone deacetylases, and chromatin remodelers. Serves as a nuclear corepressor for KRAB domain-containing zinc finger proteins (KRAB-ZFPs), mediating gene silencing by recruiting CHD3, a subunit of the nucleosome remodeling and deacetylation (NuRD) complex, and SETDB1, which methylates histone H3 at Lys-9 (H3K9me), leading to heterochromatin formation. In collaboration with SETDB1, is also required for H3K9me3 and silencing of endogenous and introduced retroviruses in a DNA- methylation independent-pathway (By similarity). Functions as a coactivator for CEBPB and NR3C1 (glucocorticoid receptor) in transcriptional activation of ORM1, and as a corepressor for ERBB4. Inhibits E2F1 activity by promoting E2F1-HDAC1 complex formation and preventing E2F1 acetylation. This contributes to CDKN1A/p21(CIP1) regulation and provides a partial backup to suppress E2F1-mediated apoptosis in the absence of RB1. Mediates the nuclear localization of several KRAB-ZFP transcription factors including KOX1, ZNF268, and ZNF300 among others. In association with isoform 2 of ZFP90, is required for the transcriptional repressor activity of FOXP3 and the suppressive function of regulatory T-cells (Treg)(PubMed:23543754). Required to maintain a transcriptionally repressive state of genes in undifferentiated embryonic stem cells (ESCs) (PubMed:24623306). Acts as a corepressor for ZFP568. Beyond its nuclear functions, acts as a positive regulator of type I interferon (IFN-I) signaling by promoting Lys-63-linked ubiquitination of TBK1, facilitating TBK1-IRF3 complex formation (PubMed:38495890). Mediates TRAF6 SUMOylation to regulate its nucleo-cytoplasmic shuttling and modulate activation of the canonical NF-kappa-B signaling pathway (PubMed:39920527). Facilitates SUMOylation of NLRP3, protecting it from 'Lys-48'-linked ubiquitination and proteasomal degradation and thereby enhancing inflammasome activation (PubMed:34373456). |
| **Cellular Location** | Nucleus. Note=Associated with centromeric heterochromatin during cell differentiation through CBX1 (By similarity). Localizes to sites of DNA damage (PubMed:25593309) {ECO:0000250\|UniProtKB:Q62318, ECO:0000269\|PubMed:25593309} |
| **Tissue Location** | Expressed in all tissues tested including spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes. |

## Background

Tripartite motif-containing 28 (TRIM28), also known as transcriptional intermediary factor 1β (TIF1β) and KAP1 (KRAB-associated protein-1), is a protein that in humans is encoded by the TRIM28 gene. The protein encoded by this gene mediates transcriptional control by interaction with the Kruppel-associated box repression domain found in many transcription factors. The protein localizes to the nucleus and is thought to associate with specific chromatin regions. KAP1 is a ubiquitously expressed protein involved in many critical functions including: transcriptional regulation, cellular differentiation and proliferation, DNA damage repair, viral suppression, and apoptosis. Its functionality is dependent upon post-translational modifications. Phosphorylation of KAP1 acts as a deactivator of the protein in many of its mechanisms while sumoylation acts as an activator.

## Images

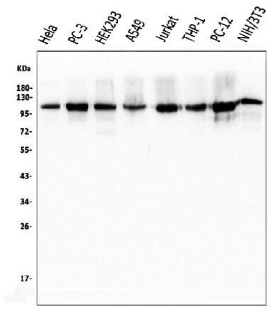



Figure 1. Western blot analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates;
Lane 2: human PC-3 whole cell lysates;
Lane 3: human HEK293 whole cell lysates;
Lane 4: human A549 whole cell lysates;
Lane 5: human Jurkat whole cell lysates;
Lane 6: human THP-1 whole cell lysates;
Lane 7: rat PC-12 whole cell lysates;
Lane 8: mouse NIH/3T3 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-KAP1/TRIM28 antigen affinity purified monoclonal antibody (Catalog # M00409-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for KAP1/TRIM28 at approximately 100KD. The expected band size for KAP1/TRIM28 is at 100KD.

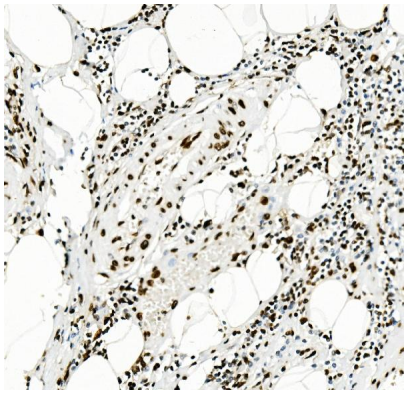


Figure 2. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1).
KAP1/TRIM28 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

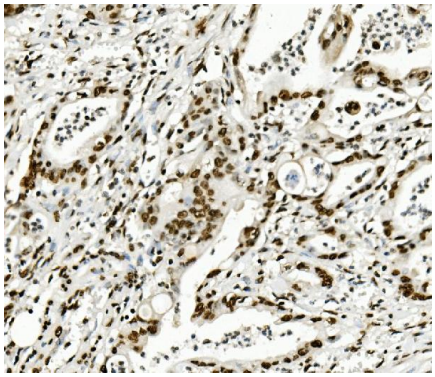


Figure 3. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

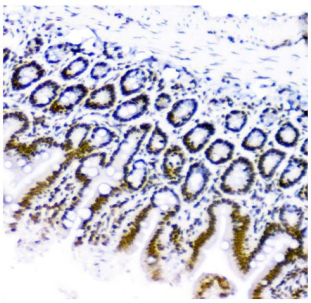


Figure 4. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of mouse small intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

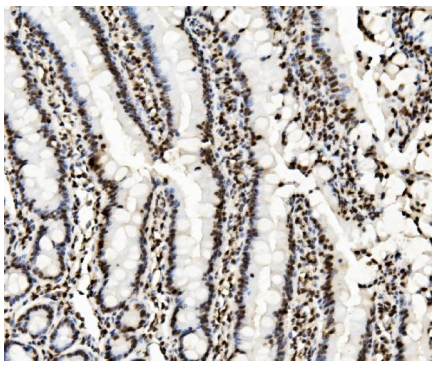


Figure 5. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

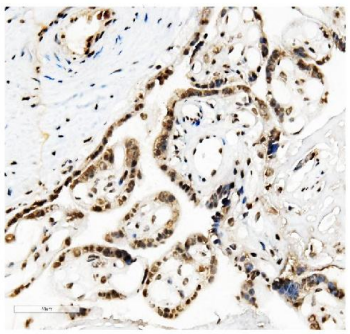
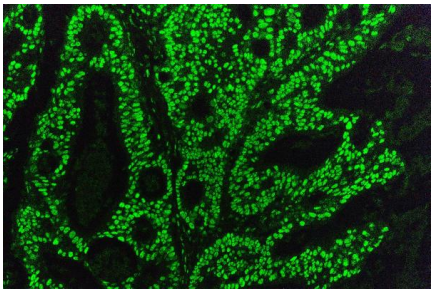


Figure 6. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

Figure 7. IF analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of human rectal carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope



retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

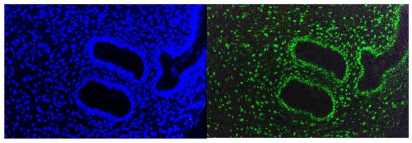


Figure 8. IF analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

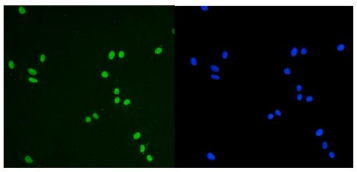


Figure 9. IF analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-KAP1/TRIM28 Antibody (M00409-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

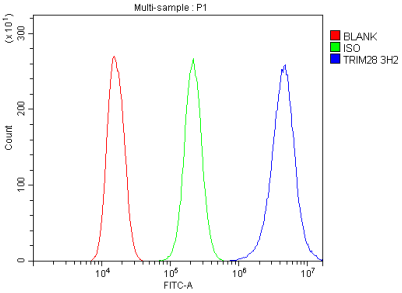



Figure 10. Flow Cytometry analysis of A549 cells using anti-KAP1/TRIM28 antibody (M00409-1). Overlay histogram showing A549 cells stained with M00409-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-KAP1/TRIM28 Antibody (M00409-1, 1 µg/1x$10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x$10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x$10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.