

**Anti-KAP1/TRIM28 Antibody Picoband™ (monoclonal, 3H2)**  
Catalog # ABO14933**Specification****Anti-KAP1/TRIM28 Antibody Picoband™ (monoclonal, 3H2) - Product Information**

Application	WB, IHC, IHC-F, IF, ICC, FC
Primary Accession	<a href="#">Q13263</a>
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.

**Anti-KAP1/TRIM28 Antibody Picoband™ (monoclonal, 3H2) - Additional Information**

**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](http://www.genenames.org/cgi-bin/gene_symbol_report?hgnc_id=16384)), KAP1, RNF96, TIF1B

**Calculated MW**

100 kDa KDa

**Application Details**

Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat  
Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, Mouse, Rat  
Immunohistochemistry (Frozen Section), 0.5-1 µg/ml, Human, Rat  
Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human  
Immunofluorescence, 2 µg/ml, Human  
Flow Cytometry, 1-3 µg/1x10<sup>6</sup> 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.

**Contents**

Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na<sub>2</sub>HPO<sub>4</sub>,

0.05mg NaN<sub>3</sub>.

#### 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.**

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

Name TRIM28 ([HGNC:16384](#))

Synonyms KAP1, RNF96, TIF1B

#### Function

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 a 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. Also specifically sumoylates IRF7, thereby inhibiting its transactivation activity. Ubiquitinates p53/TP53 leading to its proteasomal degradation; the function is enhanced by MAGEC2 and MAGEA2, and possibly MAGEA3 and MAGEA6. Mediates the nuclear localization of KOX1, ZNF268 and ZNF300 transcription factors. 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:<a href="http://www.uniprot.org/citations/23543754" target="\_blank">23543754</a>). Probably forms a corepressor complex required for activated KRAS-mediated promoter hypermethylation and transcriptional silencing of tumor suppressor genes (TSGs) or other tumor-related genes in colorectal cancer (CRC) cells (PubMed:<a href="http://www.uniprot.org/citations/24623306" target="\_blank">24623306</a>). Required to maintain a transcriptionally repressive state of genes in undifferentiated embryonic stem cells (ESCs) (PubMed:<a href="http://www.uniprot.org/citations/24623306" target="\_blank">24623306</a>). In ESCs, in collaboration with SETDB1, is also required for H3K9me<sub>3</sub> and silencing of endogenous and introduced retroviruses in a DNA-methylation independent-pathway (By similarity). Associates at promoter regions of tumor suppressor genes (TSGs) leading to their gene silencing (PubMed:<a href="http://www.uniprot.org/citations/24623306" target="\_blank">24623306</a>). The SETDB1-TRIM28-ZNF274 complex may play a role in recruiting ATRX to the 3'-exons of zinc-finger coding genes with atypical chromatin signatures to establish or maintain/protect H3K9me<sub>3</sub> at these transcriptionally active regions (PubMed:<a

href="http://www.uniprot.org/citations/27029610" target="\_blank">27029610</a>).

#### 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.

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

Provided below are standard protocols that you may find useful for product applications.

- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)
- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

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

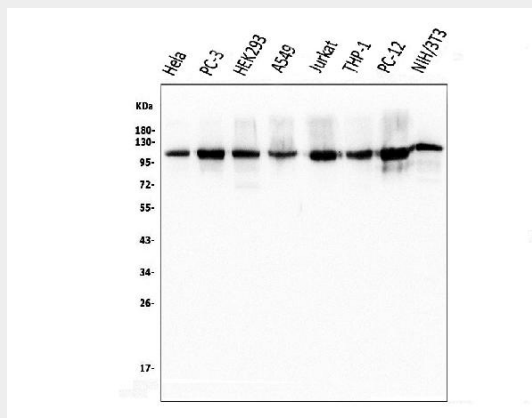


Figure 1. Western blot analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

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.

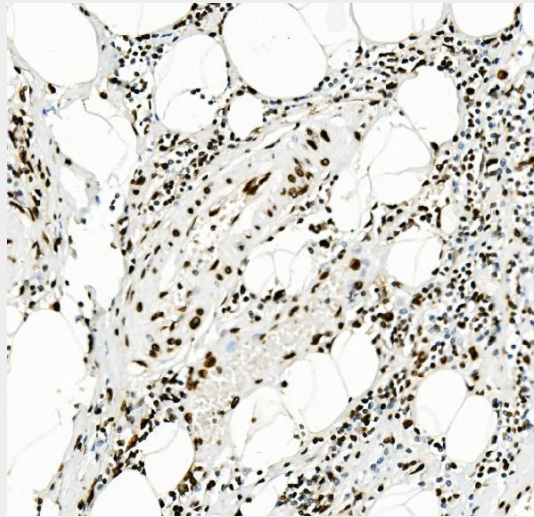


Figure 2. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

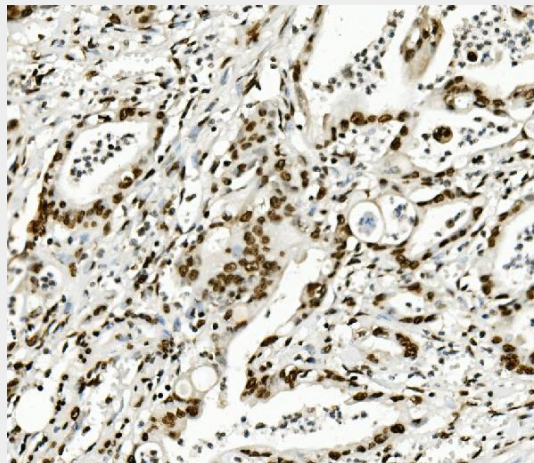


Figure 3. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

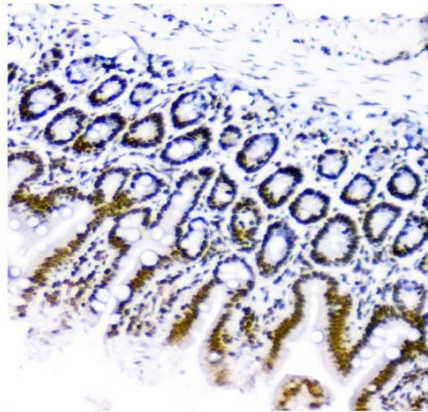


Figure 4. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of mouse small intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

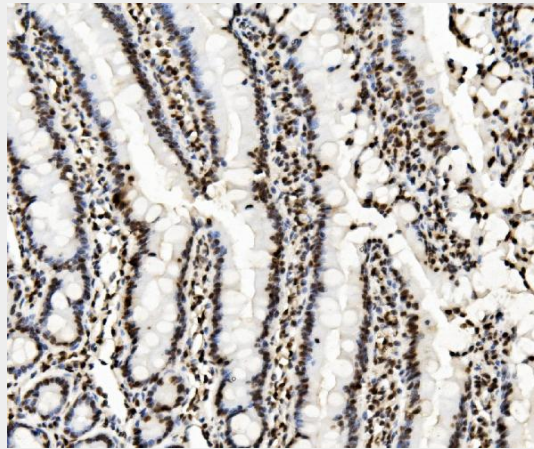


Figure 5. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

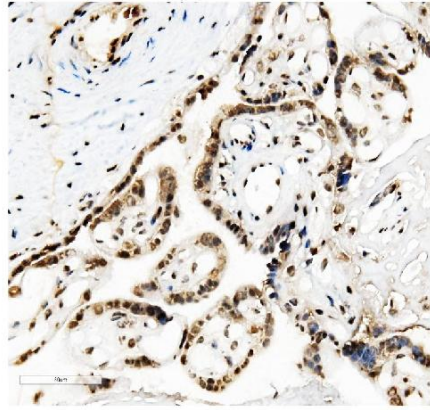


Figure 6. IHC analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu\text{g/ml}$  mouse anti-KAP1/TRIM28 Antibody (M00409-1) 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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

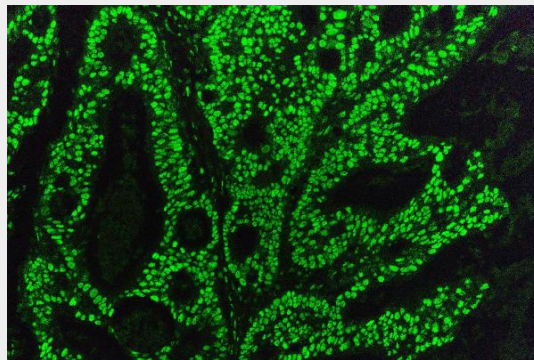


Figure 7. IF analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of human rectal carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu\text{g/mL}$  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.

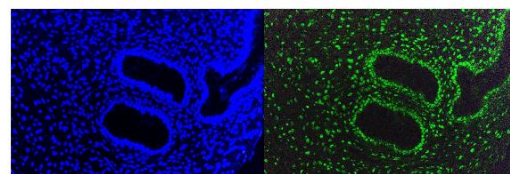


Figure 8. IF analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu\text{g/mL}$  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.

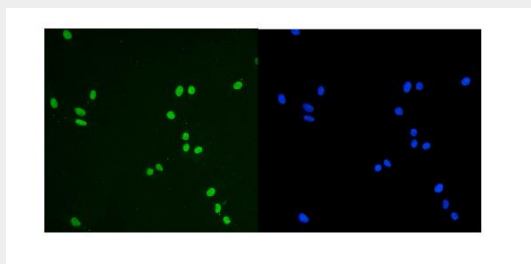


Figure 9. IF analysis of KAP1/TRIM28 using anti-KAP1/TRIM28 antibody (M00409-1). KAP1/TRIM28 was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And 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.

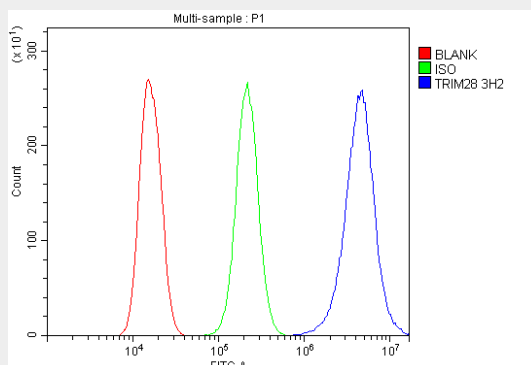


Figure 10. Flow Cytometry analysis of A549 cells using anti-KAP1/TRIM28 antibody (M00409-1). Overlay histogram showing A549 cells stained with M00409-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-KAP1/TRIM28 Antibody (M00409-1, 1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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