# Anti-Phospho-KAP1 (S824) Antibody [JE50-99]

# ET7110-11

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|---|---|
| **Product Type:** | Recombinant Rabbit monoclonal IgG, primary antibodies |
| **Species reactivity:** | Human, Mouse, Rat |
| **Applications:** | WB, IHC-P, IP, Dot Blot, IF-Cell |
| **Molecular Wt:** | Predicted band size: 89 kDa |
| **Clone number:** | JE50-99 |

**Description:** TIF1β is a member of the TIF1 (transcriptional intermediary factor 1) family, a group of transcriptional regulators that play key roles in development and differentiation. Members of this family are characterized by the presence of two conserved motifs – an N-terminal RING-B box-coiled-coil motif and a C-terminal PHD finger and bromodomain unit. TIF1β is a corepressor for KRAB (Kruppel associated box) domain containing zinc finger proteins. The KRAB domain containing zinc finger proteins are a large group of transcription factors that are vertebrate-specific, varied in their expression patterns between species, and thought to regulate gene transcription programs that control speciation. TIF1β has been shown to be essential for early embryonic development and spermatogenesis. It functions to either activate or repress transcription in response to environmental or developmental signals by chromatin remodeling and histone modification. The recruitment and association of TIF1β with heterochromatin protein (HP1) is essential for transcriptional repression, and for progression through differentiation of F9 embryonic carcinoma cells. TIF1β also plays a role in the DNA damage response. Phosphorylation of TIF1β on Ser842 occurs in an ATM-dependent manner in response to genotoxic stress and is thought to be essential for chromatin relaxation, which is in turn required for the DNA damage response.

**Immunogen:** Synthetic phospho-peptide corresponding to residues surrounding Ser824 of human KAP1.

**Positive control:** HeLa treated with 20μM Etoposide for 2 hours cell lysate, HeLa treated with 100μM Etoposide for 4 hours cell lysate, C6 treated with 25μM Etoposide for 5 hours cell lysate, NIH/3T3 treated with 25μM Etoposide for 5 hours cell lysate, HeLa cells treated with 25μM Etoposide for 5 hours, NIH/3T3 cells treated with 25μM Etoposide for 5 hours, human tonsil tissue, human breast tissue, human gastric carcinoma tissue.

**Subcellular location:** Nucleus.

**Database links:** SwissProt: Q13263 Human | Q62318 Mouse | O08629 Rat

**Recommended Dilutions:**

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|---|---|
| **WB** | 1:500-1:2,000 |
| **IHC-P** | 1:50-1:200 |
| **IP** | 1:10-1:50 |
| **Dot Blot** | Use at an assay dependent concentration. |
| **IF-Cell** | 1:100 |

**Storage Buffer:** 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

**Purity:** Protein A affinity purified.

## Images

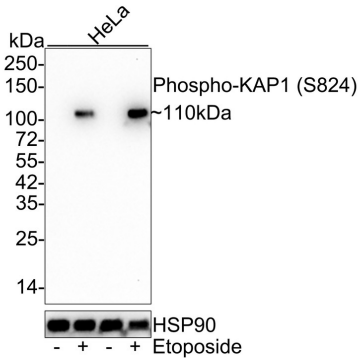


**Fig1:** Western blot analysis of Phospho-KAP1 (S824) on different lysates with Rabbit anti-Phospho-KAP1 (S824) antibody (ET7110-11) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 20μM Etoposide for 2 hours cell lysate
Lane 3: HeLa cell lysate
Lane 4: HeLa treated with 100μM Etoposide for 4 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 89 kDa
Observed band size: 110 kDa

Exposure time: 1 minute 58 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7110-11) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

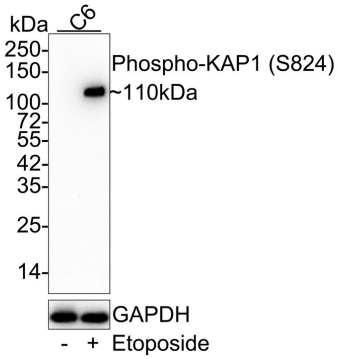


**Fig2:** Western blot analysis of Phospho-KAP1 (S824) on different lysates with Rabbit anti-Phospho-KAP1 (S824) antibody (ET7110-11) at 1/1,000 dilution.

Lane 1: C6 cell lysate
Lane 2: C6 treated with 25μM Etoposide for 5 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 89 kDa
Observed band size: 110 kDa

Exposure time: 1 minute;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7110-11) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

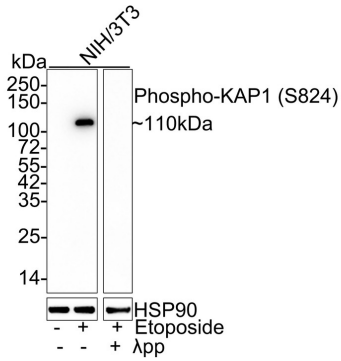


**Fig3:** Western blot analysis of Phospho-KAP1 (S824) on different lysates with Rabbit anti-Phospho-KAP1 (S824) antibody (ET7110-11) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 treated with 25μM Etoposide for 5 hours cell lysate
Lane 3: NIH/3T3 treated with 25μM Etoposide for 5 hours cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 μg/Lane.

Predicted band size: 89 kDa
Observed band size: 110 kDa

Exposure time: 1 minute 7 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET7110-11) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

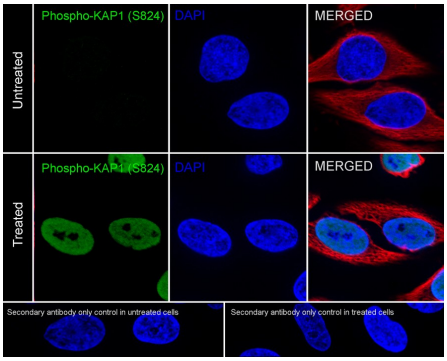


**Fig4:** Immunocytochemistry analysis of HeLa cells treated with or without 25μM Etoposide for 5 hours labeling Phospho-KAP1 (S824) with Rabbit anti-Phospho-KAP1 (S824) antibody (ET7110-11) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-KAP1 (S824) antibody (ET7110-11) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

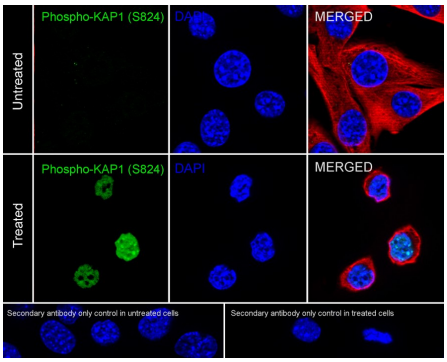


**Fig5:** Immunocytochemistry analysis of NIH/3T3 cells treated with or without 25μM Etoposide for 5 hours labeling Phospho-KAP1 (S824) with Rabbit anti-Phospho-KAP1 (S824) antibody (ET7110-11) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-KAP1 (S824) antibody (ET7110-11) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

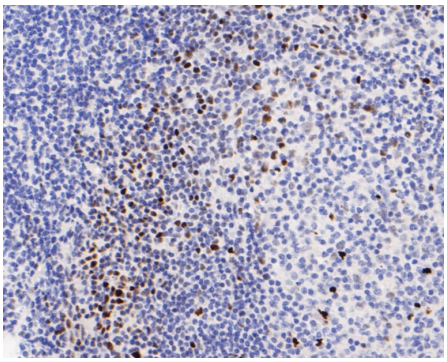

**Fig6:** Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Phospho-KAP1 (S824) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (ET7110-11, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

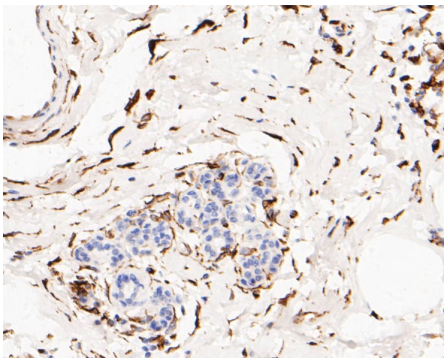

**Fig7:** Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-Phospho-KAP1 (S824) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (ET7110-11, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

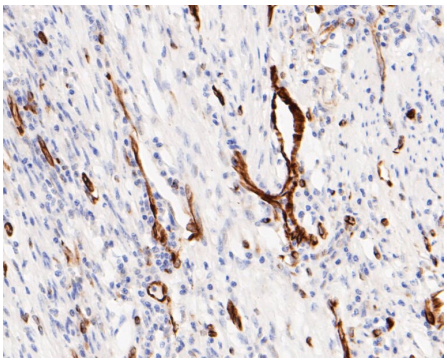

**Fig8:** Immunohistochemical analysis of paraffin-embedded human gastric carcinoma tissue using anti-Phospho-KAP1 (S824) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with dd$H_2O$ and PBS, and then probed with the primary antibody (ET7110-11, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



## Background References

1. Ziv Y. et. al. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. Nat. Cell Biol. 8:870-876(2006).
2. Li X. et. al. SUMOylation of the transcriptional co-repressor KAP1 is regulated by the serine and threonine phosphatase PP1. Sci. Signal. 3:RA32-RA32(2010).