

Acetyl-Histone H3 Antibody Sampler Kit

HAK21081



Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Histone H3 [ET1701-64]	20μl	WB, IF-Cell, IF-Tissue, IHC-P, ChIP, IP	H, M, R	15 kDa
Histone H3 (acetyl K9) [HA722132]	20μl	WB, IF-Cell, IHC-P, IF-Tissue, FC, ChIP, Dot Blot, IP	H, M, R	15 kDa
Histone H3 (acetyl K14) [ET1706-28]	20μl	WB, IF-Cell, IF-Tissue, IHC-P, IP, SNAP-ChIP, CUT&Tag-seq	H, M, R	15 kDa
Histone H3 (acetyl K18) [HA600090]	20μl	WB, IHC-P, ChIP	H, R	15 kDa
Histone H3 (acetyl K27) [HA600047]	20μl	WB, IF-Cell, IHC-P, FC, ChIP	H, M, R	15 kDa
Histone H3 (acetyl K56) [ET1608-9]	20μl	WB, IF-Cell, IF-Tissue, IHC-P, ChIP, CUT&Tag-seq	H, M, R	15 kDa
HRP-Goat [HA1001] Anti-Rabbit IgG (H+L)	100μl	WB, ELISA, IHC-P	Rab	
HRP-Goat [HA1006] Anti-Mouse IgG (H+L)	100μl	WB, ELISA, IHC-P	M	

Description: The Acetyl-Histone H3 Antibody Sampler Kit provides a fast and economical means of evaluating the acetylation sites on Histone H3. The kit contains enough primary and secondary antibodies to perform two Western mini-blot experiments.

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

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

Background Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin. The amino-terminal tails of core histones undergo various posttranslational modifications, including acetylation, phosphorylation, methylation, and ubiquitination.

Histone H3 is primarily acetylated at Lys9, 14, 18, 23, 27, and 56. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms. Phosphorylation at Ser10, Ser28, and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis. Phosphorylation at Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation at Thr3 of H3 in prophase and its dephosphorylation during anaphase.

Database links: UniProt ID: P68431, P84243, Q16695, Q6NXT2, Q71DI3, P68433, P84228, Q6LED0, P68431human, P84243human, Q16695human, Q6NXT2human, Q71DI3human, P68433mouse, P84228mouse, Q6LED0rat, P68431, P68433, Q6LED0, P68431, P84243, Q16695, Q6NXT2, Q71DI3, Q6LED0, P68431, P84243, Q16695, Q6NXT2, Q71DI3, P68433, Q6LED0, P68431, P84243, Q71DI3, P68433, Q6LED0

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Orders: 0086-571-88062880

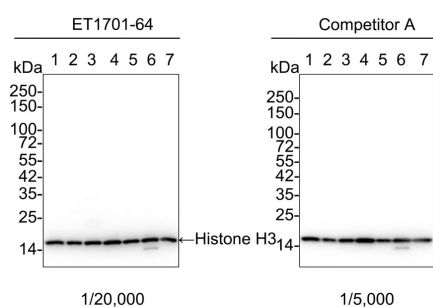
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Images

Fig1: Western blot analysis of Histone H3 on different lysates with Rabbit anti-Histone H3 antibody (ET1701-64) at 1/20,000 dilution and competitor's antibody at 1/5,000 dilution.



Lane 1: HeLa cell lysate
 Lane 2: A549 cell lysate
 Lane 3: HT-29 cell lysate
 Lane 4: HEK-293 cell lysate
 Lane 5: C2C12 cell lysate
 Lane 6: L-929 cell lysate
 Lane 7: C6 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 15 kDa

Observed band size: 15 kDa

Exposure time: 18 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ET1701-64) at 1/20,000 dilution and competitor's antibody at 1/5,000 dilution were used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

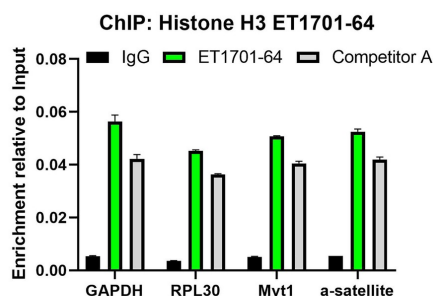


Fig2: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H3 (ET1701-64) / Competitor's antibody / Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

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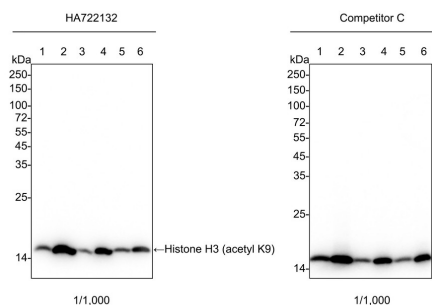
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Fig3: Western blot analysis of Histone H3 (acetyl K9) on different lysates with Rabbit anti-Histone H3 (acetyl K9) antibody (HA722132) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.



Lane 1: HeLa cell lysate
 Lane 2: HeLa treated with 500ng/mL TSA for 4 hours cell lysate
 Lane 3: NIH/3T3 cell lysate
 Lane 4: NIH/3T3 treated with 400nM TSA for 18 hours cell lysate
 Lane 5: C6 cell lysate
 Lane 6: C6 treated with 1μM TSA for 18 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 15 kDa
 Observed band size: 15 kDa

Exposure time: 6 seconds; ECL: K1801;
 4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA722132) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

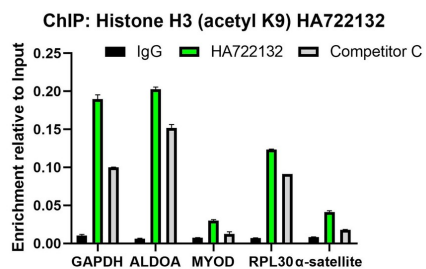


Fig4: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H3 (acetyl K9) (HA722132) / Competitor's antibody / Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

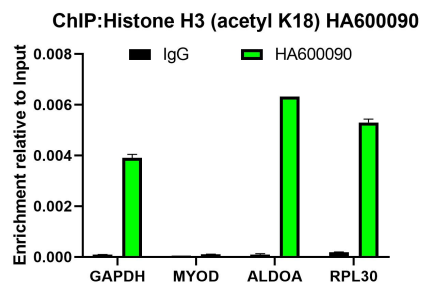


Fig5: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H3 (acetyl K9) (HA722132) / Competitor's antibody / Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

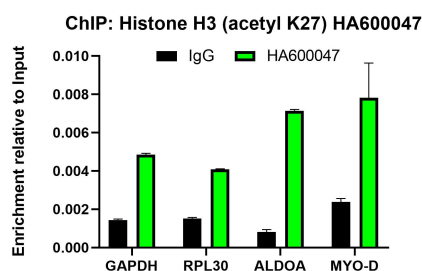
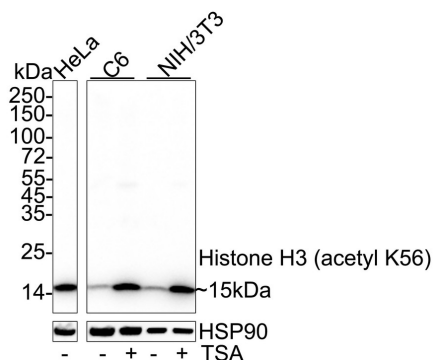


Fig6: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with 500ng/mL TSA for 4 hours with Histone H3 (acetyl K27) (HA600047) or Normal Mouse IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Fig7: Western blot analysis of Histone H3 (acetyl K56) on different lysates with Rabbit anti-Histone H3 (acetyl K56) antibody (ET1608-9) at 1/1,000 dilution.



Lane 1: HeLa cell lysate
 Lane 2: C6 cell lysate
 Lane 3: C6 treated with 1μM TSA for 18 hours cell lysate
 Lane 4: NIH/3T3 cell lysate
 Lane 5: NIH/3T3 treated with 400nM TSA for 18 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 15 kDa

Observed band size: 15 kDa

Exposure time: 14 seconds; ECL: K1801;

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 (ET1608-9) at 1/1,000 dilution was used in 5% NFDm/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

ChIP: Histone H3 (acetyl K56) ET1608-9

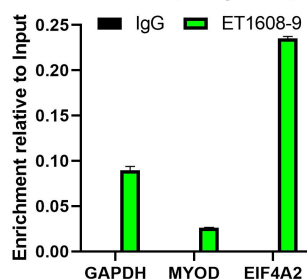


Fig8: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with Histone H3 (acetyl K56) (ET1608-9) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

Background References

1. Dai J, Sultan S, Taylor SS, Higgins JM. The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. *Genes Dev.* 2005 Feb 15;19(4):472-88.
2. Preuss U, Landsberg G, Scheidtmann KH. Novel mitosis-specific phosphorylation of histone H3 at Thr11 mediated by Dlk/ZIP kinase. *Nucleic Acids Res.* 2003 Feb 1;31(3):878-85.
3. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. *Cell.* 2000 Oct 13;103(2):263-71.

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