



Acetylated Histone/p53-K382 Monoclonal Antibody (Clone TM-5C5)

100 µg (1 mg/ml x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
TM-5C5	WB, IF, E	H, M, R	14.4-21.5 kDa (Histones) 53 kDa (p53)	Mouse IgG1

Background: Acetylation and deacetylation of nucleosomal histones play an important role in the modulation of chromatin structure, chromatin function and in the regulation of gene expression. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are two opposing classes of enzymes, which tightly control the equilibrium of histone acetylation. An imbalance in the equilibrium of histone acetylation has been associated with carcinogenesis and cancer progression. So far, a number of structurally distinct classes of compounds have been identified as HDAC inhibitors including the short-chain fatty acids, hydroxamates, cyclic tetrapeptides and benzamides. These compounds lead to an accumulation of acetylated histone proteins both in tumor cells and in normal tissues. HDAC inhibitors are able to activate differentiation, to arrest the cell cycle in G1 and/or G2, and to induce apoptosis in transformed or cancer cells. Attention is currently being drawn to molecular mechanisms involving histone deacetylases. An induction of p21WAF1 and a suppression of angiogenic stimulating factors have been observed in tumor cells following exposure to HDAC inhibitors. In xenograft models, several HDAC inhibitors have demonstrated antitumor activity with only few side effects. Several clinical trials showed that HDAC inhibitors in well-tolerated doses have significant antitumoral activities. A combination of HDAC inhibitors with differentiation-inducing agents and cytotoxic drugs is an innovative therapeutic strategy that carries the potential for significant improvements in the treatment of cancer.

Specificity/Sensitivity: The Acetylated Histone/p53-K382 Monoclonal Antibody (TM-5C5) detects endogenous acetylated Histones and several acetylated protein including acetylated p53 (acetylated lysine 382). The antibody does not recognize non-acetylated Histone nor non-acetylated p53.

Source/Purification: The monoclonal antibody is produced by immunizing mouse with a synthetic acetylated (KLH coupled) corresponding to residues surrounding Lys382 of human p53. IgG is purified by protein A-Sepharose affinity chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 µg/mL, Immunofluorescence: 5-10 µg/mL, ELISA: 0.5-1 µg/mL

Storage: Supplied in 10 mM phosphate buffer (pH 7.2), 150 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western IP: Immunoprecipitation IF: Immunofluorescence IHC: Immunohistochemistry IC: Immunocytochemistry F: Flow cytometry E: ELISA FP: Fluorescence Polarization assay

Species Cross-Reactivity Key: H: human M: mouse R: rat Hm: hamster Mk: monkey Mi: mink C: chicken X: *Xenopus* Z: zebra fish All: all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Selected Application References:

- 1) Davie, J. R & Chadee, D. N. J. Cell Biochem. (Suppl.) 30-31, 203-213, 1998
- 2) Kouzarides, T. Curr. Opin. Genet. Dev. 9, 40-84, 1999
- 3) Fenrick, R. & Hiebert, S.W. J. Cell Biochem. (Suppl.) 30-31, 194-202, 1998
- 4) Yoshida, M., Horinouchi, S. & Beppu, T. Bioassays 17, 423-430, 1995
- 5) Richon, V. M. et al. Proc. Natl.Acad. Sci. USA 93, 5705-5708, 1996
- 6) Richon, V. M. et al. Proc. Natl.Acad. Sci. USA 95 3003-3007, 1998
- 7) Cohen, L. et al. Proc. AACR 39, 108, abstr. 736, 1998
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- 9) Laherty, C. D., Yang, W-M. et al Cell 89, 349-356, 1997
- 10) Hassig, C., Fleischer, T. C. et al Cell 89, 341-347, 1997
- 11) Hoffmann, K., Grosch, G. & Jung, M Nucleic Acids Res. 27, 2057-2058, 1999

Fig. 1 Immunofluorescence analysis.

Immunofluorescence detection of Acetylated-Histone in MRC5 cells after treatment of 1 μ M Trichostatin A or vehicle (DMSO) for 4 hours using the Acetylated Histone/p53-K382 Monoclonal Antibody (TM-5C5).

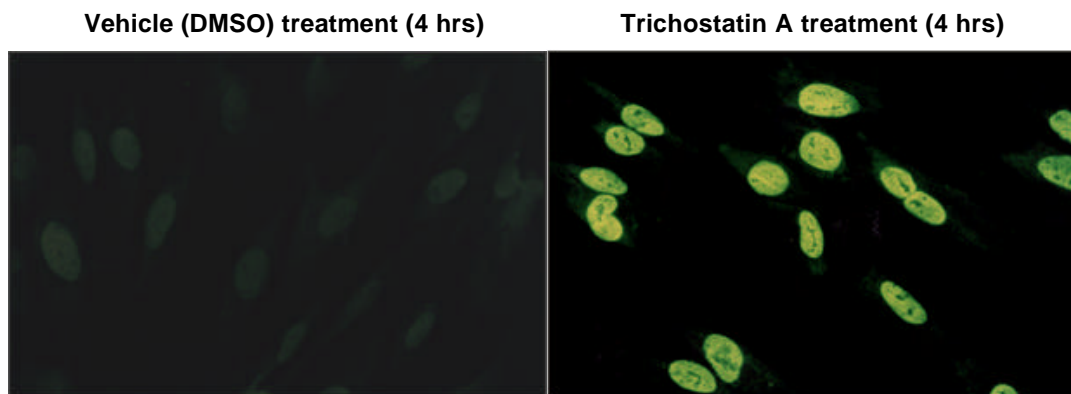


Fig. 2 ELISA for measuring level of cellular histone acetylation after treatment of Trichostatin A using the Acetylated Histone/p53-K382 Monoclonal Antibody (TM-5C5).

Typical result of time course experiment using MCF7, a breast cancer cell line, treated with 0.25 μ M Trichostatin A.

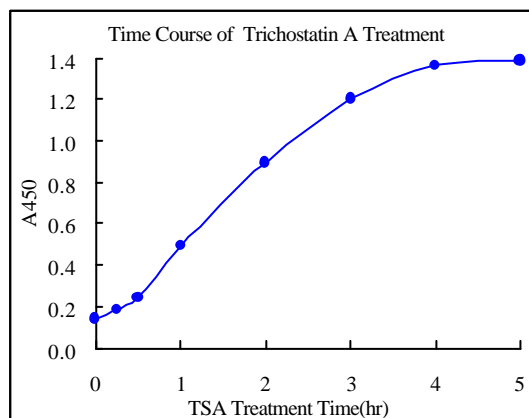


Fig. 3 Western blot analysis

Western blot analysis of extracts from MRC5 cells or Saos-2 cells that had been treated with 10 μ M Cycloheximide or 1 μ M Trichostatin A for 12 hours using the Acetylated Histone/p53-K382 Monoclonal Antibody (TM-5C5)

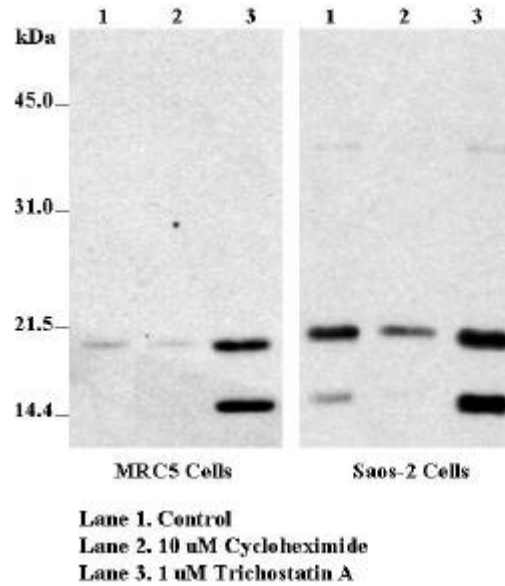
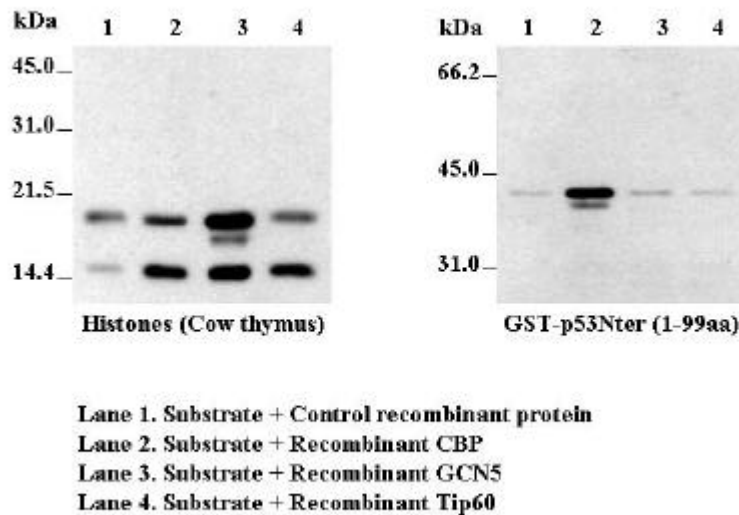


Fig. 4 *in vitro* acetylation by recombinant histone acetyltransferases (HATs)

Western blot analysis of *in vitro* acetylation of purified Histones and GST-p53Cter as substrates by recombinant histone acetyltransferases (HATs) using the Acetylated Histone/p53-K382 Monoclonal Antibody (TM-5C5)



Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red

Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).

10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).

Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 μ l Tween-20 (100%).

Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)

Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 μ l per well of 6-well plate or 500 μ l per plate of 10 cm² plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 μ l sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 μ l onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 ml of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 ml of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 ml ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten second exposure should indicate the proper exposure time.

Immunofluorescence analysis protocol

1. Grow cells to a density of 1×10^6 cells/ml on cover slip.
2. Treat cells with 0.5 μ M Trichostatin A for 1 to 6 hours.
3. Wash cells twice time with TBS
4. Add fixing agent 95% ethanol for five minutes.
5. Add blocking buffer (3% BSA TBS) and block for 15 minutes.
6. Add 2 μ g/ml of Acetylated Histone and p53-K382 Monoclonal Antibody (TM-5C5) in blocking buffer for 30 minutes.
7. Wash with TBS three times for each 2 minutes.
8. Add secondary antibody in blocking buffer (2 μ g/ml of goat anti-mouse IgG conjugated with FITC from MBL was used). Incubate in the dark, 30 minutes.
9. Wash three times with TBS.
10. Mount cells on slides. Observe cells under light of wavelength which is appropriate for the fluorescent secondary conjugate.

Related Products

* **CY-1140 Cellular Histone Acetylation Kit**

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