

## Product Information

### ANTI-ACETYLATED PROTEINS

Developed in Rabbit  
Affinity Isolated Antibody

Product Number **A 5463**

#### Product Description

Anti-Acetylated Proteins is developed in rabbit using a synthetic acetylated peptide corresponding to the N-terminus of human histone H4 (amino acids 1-20, with N- $\epsilon$ -acetylated lysines at positions 5, 8, 12, and 16), conjugated to keyhole limpet hemocyanin (KLH) as immunogen. This sequence is identical in histone H4 of many species, (i.e.: mouse, rat, *Xenopus*, chicken, *S. cerevisiae*, *S. pombe*, *C. elegans*) and not found in other histones. The antibody is affinity-purified using the immunogenic peptide immobilized on agarose.

Anti-Acetylated Proteins recognizes several acetylated proteins including acetylated histone H4 (12 kDa), acetylated H1 and H2b and acetylated bovine serum albumin (BSA). The antibody does not recognize acetylated histone H3. Applications include immunoblotting and immunocytochemistry (immunofluorescence staining of methanol/acetone fixed cells). Staining of histone H4 in immunoblotting is specifically inhibited with acetylated histone H4 immunizing peptide (human, amino acids 1-20 with N- $\epsilon$ -acetylated lysines at positions 5, 8, 12, and 16). No inhibition with the non-acetylated histone H4 peptide (human, amino acids 1-20).

The organization of chromatin into higher order structures is required for chromosome function and epigenetic gene regulation.<sup>1</sup> Nucleosomal histones H2a, H2b, H3, and H4 form octameric core histone around which DNA winds. The conserved N-terminal tails of histones, particularly of H4 and H3, have been functionally characterized and are targets for both acetylation, phosphorylation, and methylation.<sup>1-5</sup> These modifications are thought to regulate chromatin structure, histone deposition accompanying DNA replication, mitosis, and DNA repair.

Protein acetylation on lysine is an important reversible protein modification controlling protein activity. Histone acetylation at N- $\epsilon$ -lysine residues, destabilizes the

nucleosome structure, through charge neutralization, and renders the DNA more accessible to transcription factors. The level of histone acetylation correlates directly with localized transcriptional activity of a particular domain.

Histones are acetylated by histone acetyltransferases (HATs), including Gcn5p, PCAF, p300/CBP, and TAF<sub>II</sub>250,<sup>6-7</sup> and are deacetylated by histone deacetylases (HDACs).<sup>8</sup> Several proteins which are transcriptional co-activators are also HATs, while several transcriptional repressors are associated with HDACs.

Protein acetylation regulates the activity of certain transcription factors including p53, GATA-1, E2F, TFIIIE $\beta$ , TFIIIF, and EKLF.<sup>9,10</sup> In response to UV or  $\gamma$ -irradiation, the tumor suppressor p53 becomes a target for acetylation by the transcription factors p300/CBP and PCAF, which also function as co-activators for p53-mediated transcriptional activation.<sup>9,11</sup>

#### Reagent

The product is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

#### Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

#### Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

### Product Profile

A minimum working dilution of 1:1,000 is determined by immunoblotting using a whole cell extract of human epitheloid carcinoma HeLa cell line, treated with sodium butyrate.

A minimum working dilution of 1:1,000 is determined by indirect immunofluorescent staining of methanol fixed chicken fibroblast cells.

A minimum working dilution of 1:1,000 is determined by dot blot immunoassay using acetylated proteins.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

### References

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