Antibody: H3K36me3

Vendor: Abcam

Part number: ab9050

Lot number: GR52625-1

Broad Alias: PchAb 414

Test date: November 2010

User: Supplied by vendor

Procedure:

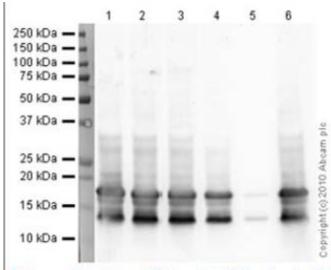
Calf Thymus Histone Preparation Nuclear Lysate was fractionated using an SDS/PAGE gel and transferred for immunoblotting. The immunoblot was cut into strips and exposed to the test antibody in the presence of the indicated synthetic peptides. Lane 1 simply illustrates the migration of the protein recognized by the antibody, and demonstrates that said protein has an apparent molecular weight consistent with the antibody recognizing a form of Histone H3. This serves as a **primary validation** of the antibody. In addition, lanes 2-6were prepared by hybridizing the immunoblot in the presence of a competing synthetic peptide. Only the peptide used in Lane 5 (H3K36me3) demonstrated inhibition of binding, consistent with the intended specificity of this antibody. We hypothesize that the lower molecular weight bands are the proteolytic byproduct of the upper band, and not other proteins, since the peptide inhibition in lane 5 serves to block reactivity to all bands.

Primary ab concentration: 1 ug / ml

Secondary antibody: Goat anti rabbit ab65484 at

1:3000

Abcam 9050 lot GR52625-1 (AP775914), Passed November 2010.



All lanes: Anti-Histone H3 (tri methyl K36) antibody - ChIP Grade (ab9050) at 1 µg/ml

Lane 1: Calf Thymus Histone Preparation Nuclear Lysate (ab121)

Lane 2: Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Histone H3 peptide (ab41430) at 0.5 µg/ml

Lane 3: Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (mono methyl K36) peptide (ab1783) at 0.5 µg/ml Lane 4: Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (di methyl K36) peptide (ab1784) at 0.5 µg/ml

Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K36) peptide (ab1784) at 0.5 µg/ml

Lane 6: Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K37) peptide (ab24417) at 0.5 µg/ml

Lysates/proteins at 0.5 µg per lane.

Secondary

Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) (ab65484) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 90 seconds