Antibody: H3K4me3 Vendor: Abcam Part number: ab8580 Lot number: 376637 Broad Alias: PchAb 91-V

Test date:October 2007User:Supplied by vendor

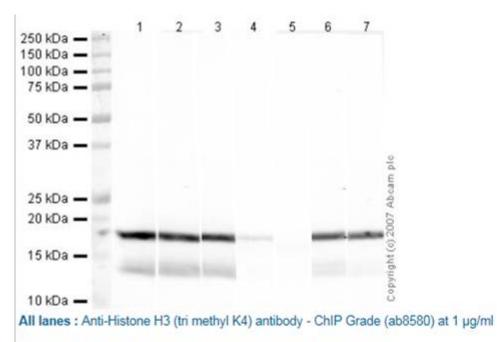
Procedure:

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 – 7 were prepared by hybridizing the immunoblot in the presence of a competing synthetic peptide. Only the peptide used in Lane 4 and 5 (land 4 to a lesser extent) demonstrated inhibition of binding, consistent with the intended specificity of this antibody for H3K4me3 and H3K4me2.

Primary ab concentration: 1 ug / ml

Secondary antibody: Rabbit IgG ab28446 at 1:10,000



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

Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (unmodified) peptide (ab7228) at 0.5 µg/ml Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (mono methyl K4) peptide (ab1340) at 0.5 µg/ml Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (di methyl K4) peptide (ab7768) at 0.5 µg/ml Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K4) peptide (ab1342) at 0.5 µg/ml Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K4) peptide (ab1342) at 0.5 µg/ml Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K9) peptide (ab1773) at 0.5 µg/ml Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K27) peptide (ab1782) at 0.5 µg/ml

Lysates/proteins at 0.5 µg per lane.

Secondary

Rabbit IgG secondary antibody (ab28446) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size : 15 kDa