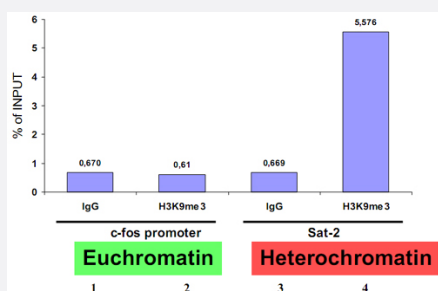


Histone H3 (K9me3) polyclonal antibody

Catalog # PAB0654

Size 50 ug

Applications



ChIP

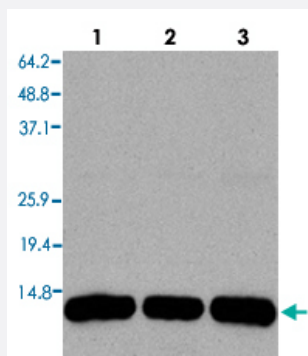
ChIP assays were performed using undifferentiated human teratocarcinoma cells (NCCIT), the Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) and optimized PCR primer sets for qPCR.

Chromatin sheared from 10,000 cells and 1 ug of Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) were used per ChIP experiment. IgG is used as negative IP control.

H3K9me3 is a marker for heterochromatin.

Therefore, we used the promoter of a house keeping gene c-fos, which is under active transcription, as negative PCR control.

SAT-2, present in heterochromatin, is used as positive PCR control.



Western Blot (Cell lysate)

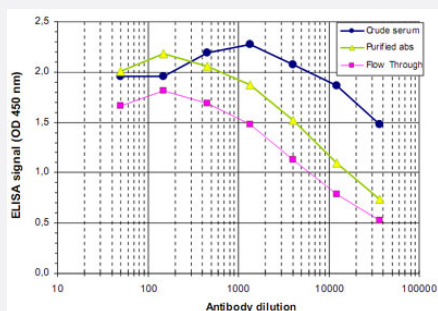
NB4 cells were treated with ATRA for 168h (Lane 1) and 24h (Lane 2) to induce cell differentiation and NB4 cells were non-treated (Lane 3). Histone (acid) extracts of treated and non-treated cells were analysed by Western blot using the Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) at a dilution of 1 : 1,000.

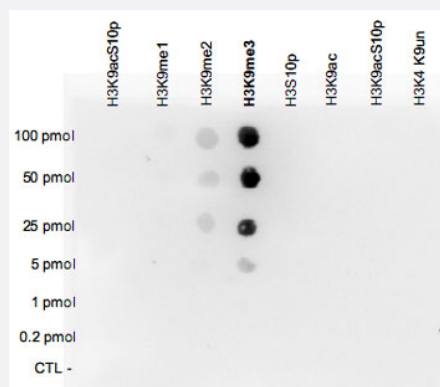
Enzyme-linked Immunoabsorbent Assay

ELISA used to determine the concentration of a primary antibody using a series of dilutions of Histone H3 (K9me3) polyclonal antibody (Cat # PAB0655), Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) and flow-through in antigen coated wells.

The antigen used in this case is the peptide including the histone modification of interest.

We plotted the absorbance versus antibody dilution to estimate the TITER : 1 : 35,000 for crude serum (Cat # PAB0655) and 1 : 2,600 for affinity purified antibody (Cat # PAB0654).





Dot Blot

Dot Blot to test the cross reactivity of the Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) with other histones and other histone modifications. Other histone modifications include mono- and dimethylation of the same lysine and mono-, di- and trimethylation of adjacent lysines.

To determine the cross reactivity, 0.2 to 100 pmol of peptide containing the respective histone modifications were spotted on a membrane.

The antibody was used at a dilution of 1 : 1,000.

Specification

Product Description	Rabbit polyclonal antibody raised against synthetic peptide of histone H3 (trimethylated lysine 9).
Immunogen	A synthetic peptide (conjugated with KLH) containing the trimethylated lysine 9 (or [K9me3]) of human histone H3.
Host	Rabbit
Reactivity	Human
Form	Liquid
Recommend Usage	The optimal working dilution should be determined by the end user.
Storage Buffer	In PBS (0.05% sodium azide, 0.05% proclin 300)
Storage Instruction	Store at -20°C. For long term storage store at -80°C. Aliquot to avoid repeated freezing and thawing.
Note	This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Applications

- **ChIP**

ChIP assays were performed using undifferentiated human teratocarcinoma cells (NCCIT), the Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) and optimized PCR primer sets for qPCR.

Chromatin sheared from 10,000 cells and 1 µg of Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) were used per ChIP experiment.

IgG is used as negative IP control.

H3K9me3 is a marker for heterochromatin.

Therefore, we used the promoter of a house keeping gene c-fos, which is under active transcription, as negative PCR control. SAT-2, present in heterochromatin, is used as positive PCR control.

- **Western Blot (Cell lysate)**

NB4 cells were treated with ATRA for 168h (Lane 1) and 24h (Lane 2) to induce cell differentiation and NB4 cells were non-treated (Lane 3). Histone (acid) extracts of treated and non-treated cells were analysed by Western blot using the Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) at a dilution of 1 : 1,000.

- **Enzyme-linked Immunoabsorbent Assay**

ELISA used to determine the concentration of a primary antibody using a series of dilutions of Histone H3 (K9me3) polyclonal antibody (Cat # PAB0655), Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) and flow-through in antigen coated wells. The antigen used in this case is the peptide including the histone modification of interest.

We plotted the absorbance versus antibody dilution to estimate the TITER : 1 : 35,000 for crude serum (Cat # PAB0655) and 1 : 2,600 for affinity purified antibody (Cat # PAB0654).

- **Dot Blot**

Dot Blot to test the cross reactivity of the Histone H3 (K9me3) polyclonal antibody (Cat # PAB0654) with other histones and other histone modifications.

Other histone modifications include mono- and dimethylation of the same lysine and mono-, di- and trimethylation of adjacent lysines.

To determine the cross reactivity, 0.2 to 100 pmol of peptide containing the respective histone modifications were spotted on a membrane.

The antibody was used at a dilution of 1 : 1,000.

Publication Reference

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- [Intra- and inter-nucleosomal protein-DNA interactions of the core histone tail domains in a model system.](#)

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