diagenode
A Hologic Company

TECHNICAL DATASHEET

H3K9me3 recombinant antibody and negative control - Premium

Cat. No. C15500003

Type: Monoclonal, recombinant **ChIP-grade/ChIP-seq grade**

Source: Fab format with human framework (Hattori T. *et al.*, 2013)

Lot #: 001

Size: 50 µg/200 µl

Concentration: 0.25 µg/µl

Specificity: Human, mouse, drosophila, yeast, wide range expected

Storage: Store at -80°C

Precautions: This product is for research use only
Not for use in diagnostic or therapeutic procedures

Description : Recombinant antibody raised against the region of histone H3 containing the trimethylated lysine 9 (H3K9me3) (ref 1). The antibody has been coupled to biotin. Also included in this package is the negative control, a non functional antibody obtained by mutating specific residues.

Applications

	Suggested dilution/amount	Results
ChIP*	0.2-1.8 µg per ChIP	Fig 1, 2
IF	1:500	Fig 3

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-5 µg per IP

This recombinant antibody has been described in:

(1) Hattori T. *et al.*, Nature Methods 10, 992–995 (2013)

Target description

Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class H2A, H2B, H3 and H4 assemble and are wrapped by 146 base pairs of DNA to form one octameric nucleosome. Histone tails undergo numerous post-translational modifications, which either directly or indirectly alter chromatin structure to facilitate transcriptional activation or repression or other nuclear processes. In addition to the genetic code, combinations of the different histone modifications reveal the so-called "histone code". Histone methylation and demethylation is dynamically regulated by respectively histone methyl transferases and histone demethylases. Trimethylation of histone H3K9 is associated with imprinted regions, satellite repeats and ZNF repeat genes.

Results

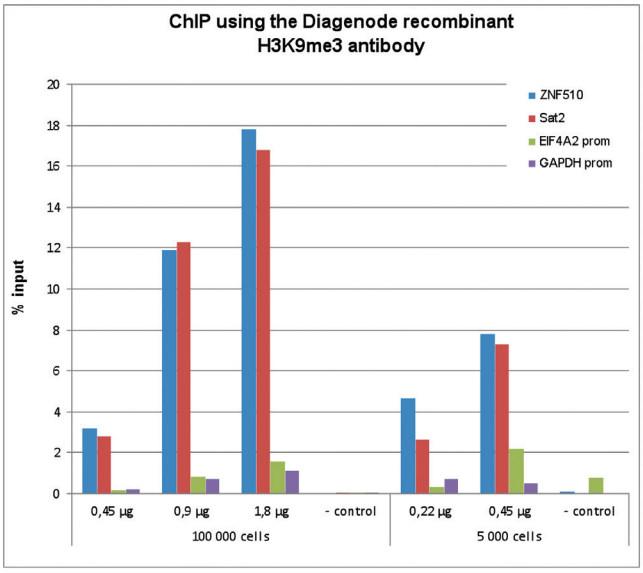
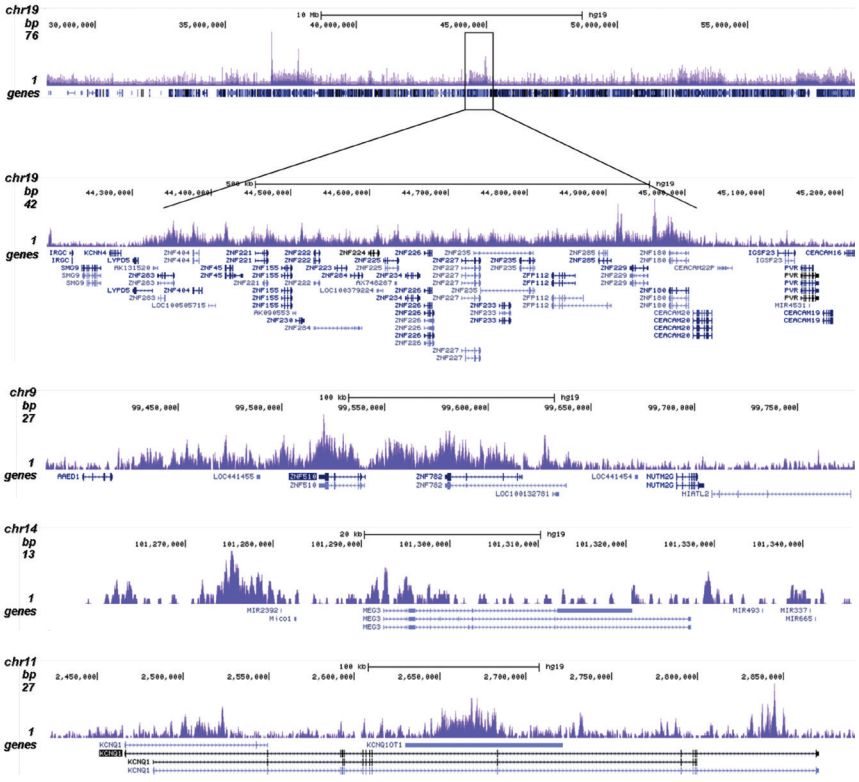


Figure 1. ChIP results obtained with the Diagenode recombinant antibody directed against H3K9me3

ChIP assays were performed using human HeLa cells, the Diagenode recombinant antibody against H3K9me3 and optimized PCR primer sets for qPCR. ChIP was performed on sheared chromatin from 100,000 and 5,000 cells with the "True MicroChIP kit (cat. No. C01010130). See page 4: Protocol for binding the recombinant H3K9me3 antibody to streptavidin-coated beads (Hattori T. *et al.*, 2013). Different amounts of the antibody were analysed. A negative control recombinant antibody 1 or 5 µg/IP) was used as negative IP control. QPCR was performed with primers for the heterochromatin marker Sat2 and for the ZNF510 gene, used as positive controls, and for the promoters of the active EIF4A2 and GAPDH genes, used as negative controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

Figure 2. ChIP-seq results obtained with the Diagenode recombinant antibody directed against H3K9me3

ChIP was performed with 1.3 µg of the Diagenode antibody against H3K9me3 on sheared chromatin from 4 million K562 cells. The IP'd DNA was analysed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The sequenced reads were aligned to human genome version 19 using the ELAND algorithm. Figure 2A shows the signal distribution along the long arm of chromosome 19 and a zoomin to an enriched region containing several ZNF repeat genes. Figure 2B shows the enrichment at ZNF510 and Figure 2 C and D show the enrichment at the MEG3 and KCNQ1 imprinted genes.



Results

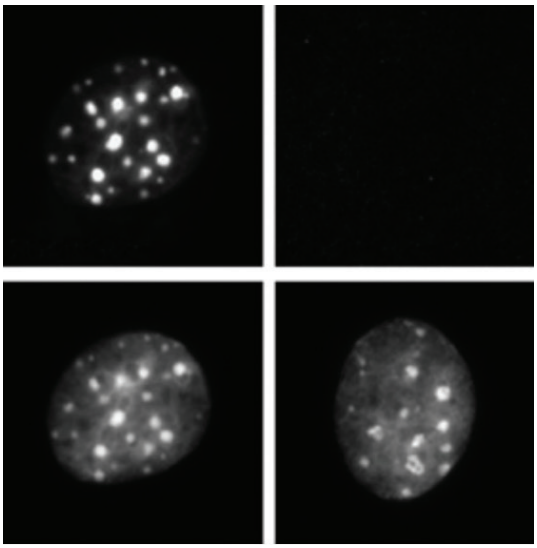


Figure 3. Immunofluorescence using the Diagenode recombinant antibody directed against H3K9me3

NIH 3T3 cells were stained with the Diagenode antibody against H3K9me3, left or with the negative control recombinant antibody, right. The bottom panel shows counterstaining of the cells with DAPI.

(Hattori T. *et al.*, 2013).

Protocol for binding the recombinant H3K9me3 antibody to streptavidin-coated beads

(Hattori T. *et al.*, 2013).

The recombinant H3K9me3 antibody (Cat. No. C15500003) has been validated in ChIP with the True MicroChIP kit (Cat. No. C01010130). However, since this antibody is a biotinylated Fab fragment, the protocol was slightly adapted. The protein A/G coated magnetic beads

included in the True MicroChIP kit were replaced by streptavidin-coated beads to capture the recombinant antibody. The protocol below is intended for binding of the antibody to streptavidin beads for one ChIP experiment. Scale up accordingly for larger numbers of ChIP experiments.

Material required

- Dynabeads M280 Streptavidin (Invitrogen)
  Alternatively Streptavidin MagneSphere paramagnetic beads (Promega) can be used
- TBS containing 0.5% BSA (called TBS/BSA in the protocol)
- Biotin. Prepare a solution of 5 µM biotin in TBS containing 0.5 % BSA
- Diamag 1.5 magnetic rack (Cat No. kch-816-015)

NOTE: Please proceed with **STEP 1** - Cell collection and DNA-protein crosslinking as well as **STEP 2** - Cell lysis and chromatin shearing, as explained in the True MicroChIP kit protocol. In **STEP 3** – Magnetic Immunoprecipitation and washes, proceed up to **point 22** for **Detailed protocol** or **point 13** for **Short protocol** (ie proceed up to chromatin dilution after the shearing and use this diluted chromatin at the end of the recombinant antibody binding protocol below).The protocol below is optimized for working with 100 000 cells. When using less cells, you should decrease the amount of antibody and beads to use.

Recombinant antibody binding protocol

NOTE: a) Biotin has low solubility in water. Prepare first 50 mM biotin solution in dimethyl sulfoxide (DMSO) and dilute it in TBS to make 100 µM biotin solution. Keep the 50 mM biotin solution in DMSO at -20°C for long-term storage, and the 100 µM biotin solution in TBS at 4°C for up to a month.
b) Do not change antibody-beads ratio as this parameter has been thoroughly optimized.

1. Suspend Streptavidin MagneSphere paramagnetic particules very well by vortexing.
2. Dispense beads in a micro-tube. Use 130 µl (or 73 µl Dynabeads) of the beads for each ChIP experiment.
3. Place the tube containing the beads suspension on the magnetic rack for 1 min, and then discard the supernatant using a pipetman. Add the same volume of TBS/BSA to the beads and suspend it by pipetting. Repeat this step once more.
4. Dispense 125 µl of the bead suspension from **point 3** (or 68 µl Dynabeads) into a regular micro tube containing 75 µl of TBS/BSA (or 132 µl if Dynabeads are used), then place the tube on the magnetic rack for 1 min (one tube is used for one ChIP experiment).
5. Dilute 1.8 µg of the biotinylated antibody with TBS/BSA so that the total volume is 105 µl.
6. Discard the supernatant of the bead suspension from **point 4**. Add 100 µl of the antibody solution from STEP5 to each tubes (DO NOT add a concentrated antibody solution directly to the beads). Rotate the tubes for 1 h at 4°C.
7. Place the tube on the magnetic rack for 1 min, and then discard the supernatant. Add 200 µl of 5 µM biotin (in TBS/BSA) and suspend beads. Rotate beads for 10 min at 4°C. Repeat this step once more.
8. It is important to block the remaining biotin-binding sites on the beads with biotin so that endogenously biotinylated proteins are not captured.
9. Place the tubes on the magnetic rack for 1 min, and then discard the supernatant. Add 200 µl of Beads Wash Buffer tBW1 (from the True MicroChIP kit) and suspend the beads. Repeat this step once more.
10. Transfer the prepared beads to new siliconized micro tubes. Place the tubes on the magnetic rack for 1 min, and then discard supernatant.
11. To each tube, add the diluted chromatin (200 µl of diluted chromatin equivalent to 100 000 cells prepared in **STEP 3** from the True MicroChIP kit protocol). Rotate tubes at 4°C for overnight.

NOTE: from here on please follow again the TrueMicroChIP kit protocol **(Short protocol :** STEP 3 – Magnetic Immunoprecipitation and washes – **point 18 / Detailed protocol STEP 3** – Magnetic Immunoprecipitation and washes **– point 28).**

References

Hattori T. *et al.*, Nature Methods 10, 992–995 (2013)

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