diagenode
Innovating Epigenetics Solutions

ChIP-seq grade

TECHNICAL DATASHEET

H3.3 monoclonal antibody

Cat. No. C15210011

Type: Monoclonal	Specificity: Human. Other species not tested.
Isotype: NA	Concentration: 1 µg/µl
Source: Rabbit	Purity: Protein A purified monoclonal antibody in PBS containing 50% glycerol, 1% BSA and 0.09% azide.
Lot No.: 001	Storage: Store at -20°C
Size: 100 µg/ 100 µl	Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Last Data Sheet Update: March 16, 2017

Description

Monoclonal antibody raised in rabbit against histone variant H3.3, using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	4 µg/ChIP	Fig 1, 2
Western Blotting	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.

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. Histone variant H3.3 is preferably present at active genes.

Diagenode sa. BELIGUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
info@diagenode.com | orders@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
info.na@diagenode.com | orders.na@diagenode.com

Validation data

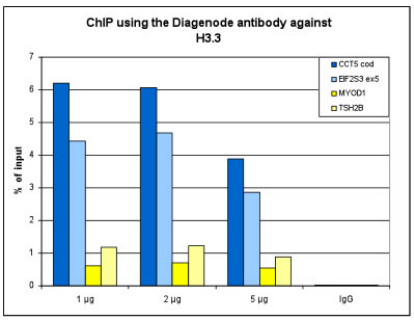
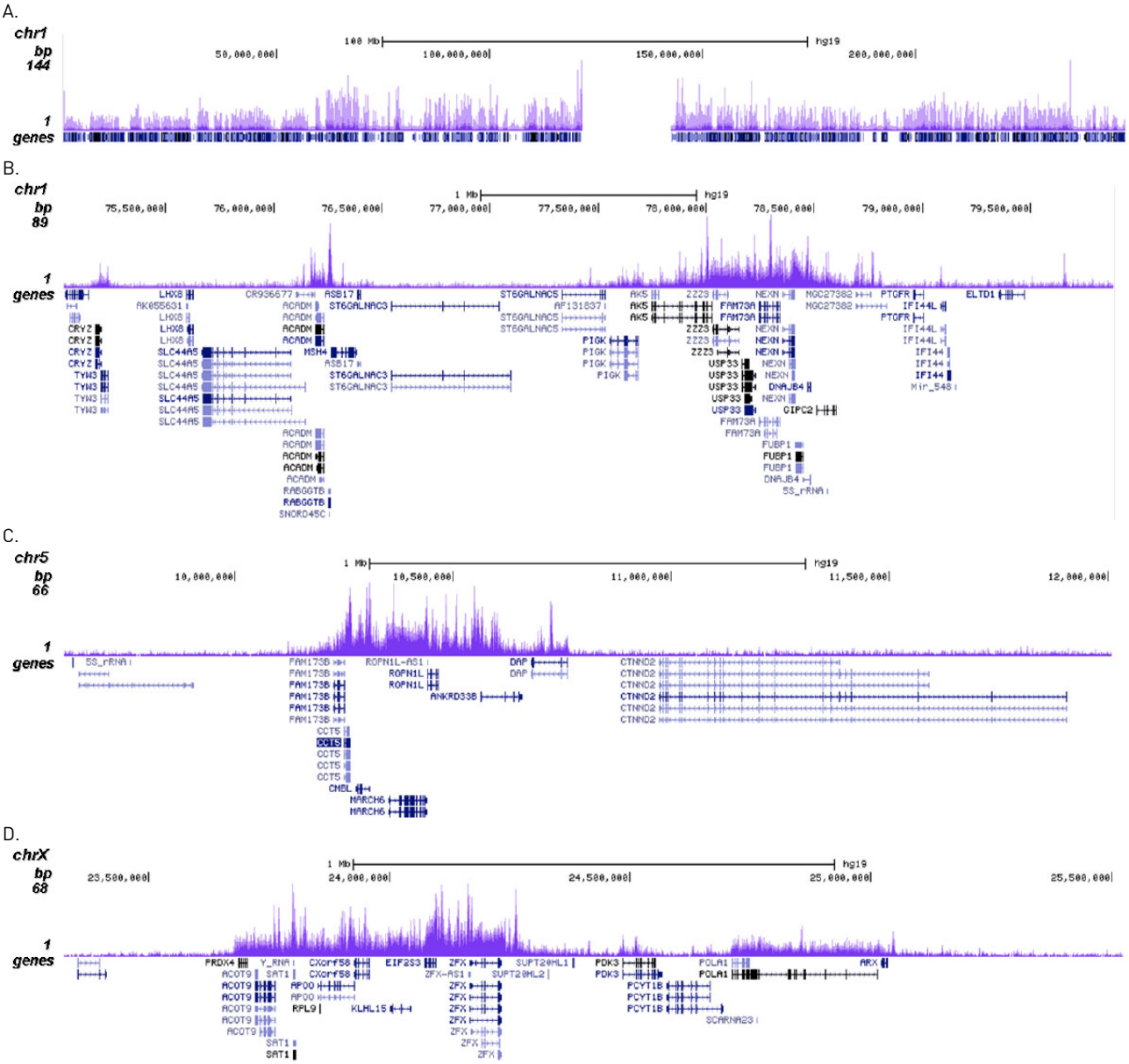


Figure 1. ChIP results obtained with the Diagenode monoclonal antibody directed against H3.3
ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3.3 (cat. No. C15210011) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (cat. No. C01010055) on sheared chromatin from 1,000,000 cells. A titration of the antibody consisting of 1, 2 and 5 µg per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the coding regions of the active CCT5 and EIF2S3 genes, used as positive controls, and for the inactive MYOD1 and TSH2B 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).



Diagenode sa. BELIGUM | EUROPE

LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
info@diagenode.com | orders@diagenode.com

Diagenode Inc. USA | NORTH AMERICA

400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
info.na@diagenode.com | orders.na@diagenode.com

Figure 2.ChIP-seq results obtained with the Diagenode monoclonal antibody directed against H3.3

ChIP was performed with 1 µg of the Diagenode antibody against H3.3 (cat. No. C15210011) on sheared chromatin from 1,000,000 HeLa cells using the "iDeal ChIP-seq" kit as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 3.5 Mb region of human chromosome 1 (figure 2A and B) and in two genomic regions surrounding the CCT5 and EIF2S3 positive control genes (figure 2C and D).

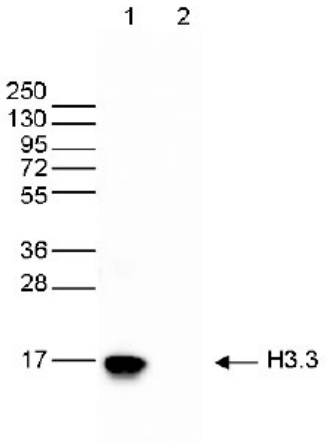


Figure 3. Western blot analysis using the Diagenode monoclonal antibody directed against H3.3

Western blot was performed on 1 µg of recombinant histone H3.1 (lane 2) and on 1 µg of recombinant histone H3.3 (lane 1) using the Diagenode monoclonal antibody against H3.3 (cat. No. C15210011) diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

Diagenode sa. BELIGUM | EUROPE

LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
info@diagenode.com | orders@diagenode.com

Diagenode Inc. USA | NORTH AMERICA

400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
info.na@diagenode.com | orders.na@diagenode.com