diagenode

Innovating Epigenetic Solutions

SIMPLY SUPERIOR EPIGENETIC ANTIBODIES

VALIDATED IN:

- ChIP and ChIP-seq
- MeDIP and MeDIP-seq
- Western blot
- RNA IP
- Immunoprecipitation
- Immunofluorescence
- Peptide array
- and more...

Unparalleled ChIP and ChIP-Seq results with rigorously validated antibodies

Since 2004, Diagenode has partnered with leading epigenetics experts and the epigenetics consortium to produce and validate epigenetic reagents including antibodies. In 2012, Diagenode revolutionized immunoprecipitation with a novel, rigorous antibody classification system for epigenetic assays. We have established a novel categorization of our antibodies into three different classes dependent on their level of validation to help you establish confidence in choosing your antibody. Finally, achieve robust results with antibodies you can TRUST.

- **Focused** — Diagenode's selection of antibodies is exclusively dedicated for epigenetic research
- **Strict quality standards** with rigorous QC and validation
- **Classified** based on level of validation for application flexibility

LEVEL OF VALIDATION

PREMIUM

VALIDATED BY CHIP-SEQ/MEDIP-SEQ + MORE!

- ✓ ChIP-seq/MeDIP-seq
- ✓ Dot blot
- ✓ Peptide array
- ✓ Western blot
- ✓ Immunofluorescence
- ✓ ELISA

CLASSIC

VALIDATED BY CHIP/CHIP-SEQ

- ✓ ChIP/ChIP-seq

or at least three of these:

- ✓ Dot blot
- ✓ Peptide array
- ✓ Western blot
- ✓ Immunofluorescence
- ✓ ELISA

PIONEER

VALIDATED BY ELISA

- ✓ ELISA

or ELISA plus one of these:

- ✓ Dot blot
- ✓ Western blot
- ✓ Immunofluorescence

* *Based on stringent Blueprint ChIP-seq quality standards as described by the European Blueprint Consortium (similar to ENCODE in the U.S.).*

Diagenode's highly validated antibodies:

- Highly sensitive and specific
- Cost-effective (requires less antibody per reaction)
- Batch-specific data is available on our website
- Expert technical support
- Sample sizes available
- 100% satisfaction guarantee

Finally, achieve robust results with antibodies you can TRUST

Our Premium antibody product line undergoes a very strict quality control process and the most stringent bioinformatic tests. All antibodies exhibit superior performance for virtually any application. A subset of these antibodies has also been validated from the extensive work from the EU community of epigenetic experts. Our partners consider these as the best possible, highest performance antibodies available and which will likely become the next international standards.

Highest level of validation on the market

Premium antibodies	Target	Cat. No.
5-mC monoclonal antibody **33D3**	5-methylcytosine	C15200081
H3K4me1 polyclonal antibody	Histone H3 containing the monomethylated lysine 4	C15410194
H3K4me3 polyclonal antibody	Histone H3 containing the trimethylated lysine 4	C15410003
H3K9me3 recombinant antibody and negative control	Histone H3 containing the trimethylated lysine 9	C15500003
H3K9me3 polyclonal antibody	Histone H3 containing the trimethylated lysine 9	C15410193
H3K9/14ac polyclonal antibody	Histone H3 containing the acetylated lysines 9 and 14	C15410200
H3K27ac polyclonal antibody	Histone H3 containing the acetylated lysine 27	C15410196
H3K27me3 polyclonal antibody	Histone H3 containing the trimethylated lysine 27	C15410195
H3K36me3 polyclonal antibody	Histone H3 containing the trimethylated lysine 36	C15410192
H4K20me3 polyclonal antibody	Histone H4 containing the trimethylated lysine 20	C15410207
H2A.Z polyclonal antibody	Histone variant H2A.Z	C15410201
H2A.Zac polyclonal antibody	Histone H2A.Z containing the acetylated lysines 5, 7 and 11	C15410202
HDAC1 polyclonal antibody	Histone Deacetylase 1	C15410325

All are polyclonal antibodies raised in rabbit against specified region using a KLH-conjugated synthetic peptide. Quantities of these highly validated antibodies are in limited supply. Please inquire about availability to your sales representative.

Check out the complete list of Diagenode's antibodies at www.diagenode.com

Customer Feedback

In life sciences, epigenetics is nowadays the most rapid developing field with new astonishing discoveries made every day. To keep pace with this field, we are in need of reliable tools to foster our research - tools Diagenode provides us with. From antibodies to automated solutions - all from one source and with robust support. Antibodies used in our lab: H3K27me3 polyclonal antibody – Premium, H3K4me3 polyclonal antibody – Premium, H3K9me3 polyclonal antibody – Premium, H3K4me1 polyclonal antibody – Premium, CTCF polyclonal antibody – Classic, Rabbit IgG.

Dr. Florian Uhle, Dept. of Anesthesiology, Heidelberg University Hospital, Germany

Stringent validation criteria

Cross-reactivity

Tested by Dot Blot and peptide arrays (specificity factor →30)

ChIP-grade

qPCR with two positive and two negative control targets, expected profile with signal-to-noise ratio → 5

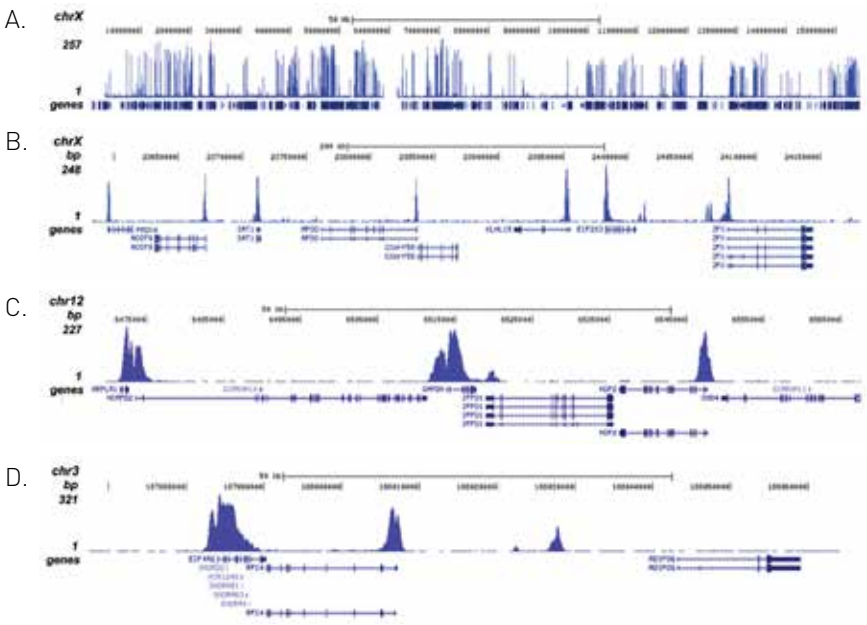
ChIP-seq grade

Extensive bioinformatic analysis with peak detection with SICER and %RIP →40; peak comparison with published data (ENCODE, Broad Institute) and overlap of the top 40 most significant peaks →80%.

Western Blot

Performed on whole cell extracts, histone extracts and recombinant histones H2A, H2B, H3 and H4. Single band of the expected size in WCE and HE - if additional bands are present at the size of other histones, the intensity should be <10% of the specific signal. Other additional bands in whole cell extracts should have intensity <20% of the specific signal. Signal with any of the recombinant histones <10% of the signal obtained with HE.

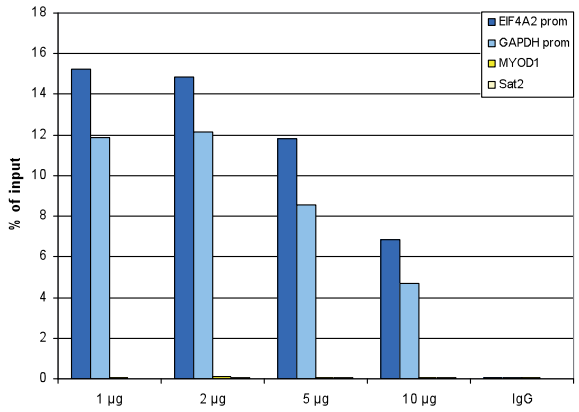
Figure 1. ChIP-seq results obtained with the Diagenode antibody directed against H3K4me3



ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 1 µg of the Diagenode antibody against H3K4me3 (Cat. No. C15410003) as described above. The IP'd DNA was subsequently analysed on an Illumina Genome Analyzer. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions.

The 36 bp tags were aligned to the human genome using the ELAND algorithm. Figure 1 shows the peak distribution along the complete sequence and a 600 kb region of the X-chromosome (figure 1A and B) and in two regions surrounding the GAPDH and EIF4A2 positive control genes, respectively (figure 1C and D). These results clearly show an enrichment of the H3K4 trimethylation at the promoters of active genes.

Figure 2. ChIP results obtained with the Diagenode antibody directed against H3K4me3



ChIP assays were performed using human HeLa cells, the Diagenode antibody against H3K4me3 (Cat. No. C15410003) and optimized PCR primer pairs for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010051), using sheared chromatin from 1 million cells. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoter of the active genes GAPDH and EIF4A2, used as positive controls, and for the inactive MYOD1 gene and the Sat2 satellite repeat, 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). These results are in accordance with the observation that trimethylation of K4 at histone H3 is associated with the promoters of active genes.

Figure 3. Cross reactivity tests using the Diagenode antibody directed against H3K4me3

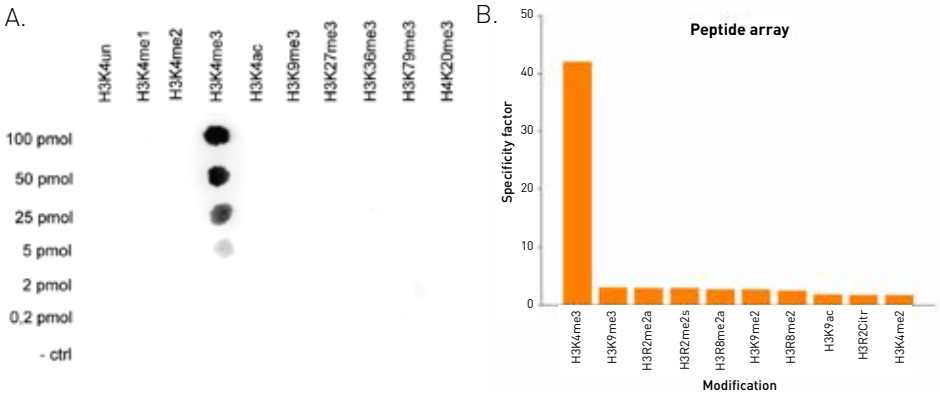
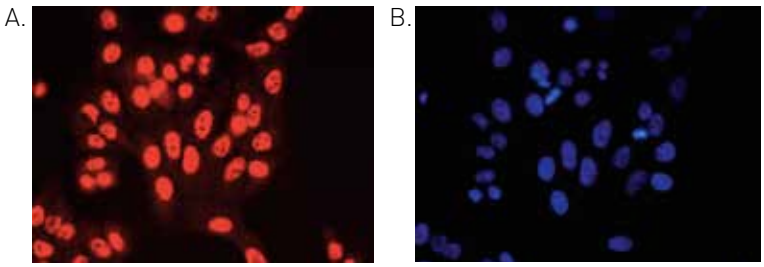


Figure 2A. *To test the cross reactivity of the Diagenode antibody against H3K4me3 (Cat. No. C15410003), a Dot Blot analysis was performed with peptides containing other histone modifications and the unmodified H3K4. One hundred to 0.2 pmol of the respective peptides were spotted on a membrane. The antibody was used at a dilution of 1:10,000. Figure 2A shows a high specificity of the antibody for the modification of interest.*

Figure 2B. *The specificity of the antibody was further demonstrated by peptide array analyses on an array containing 384 peptides with different combinations of modifications from histone H3, H4, H2A and H2B. The antibody was used at a dilution of 1:2,000. Figure 2B shows a high specificity for the peptides containing the H3K4me3 modification.*

Figure 4. Immunofluorescence using the Diagenode antibody directed against H3K4me3



Human osteosarcoma (U2OS) cells were stained with the Diagenode antibody against H3K4me3 (Cat. No.C15410003) and with DAPI. Cells were fixed with 4% formaldehyde for 20' and blocked with PBS/TX-100 containing 5% normal goat serum. The cells were immunofluorescently labeled with the H3K4me3 antibody (top) diluted 1:200 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa568 or with DAPI (bottom), which specifically labels DNA.

Epigenetic Antibodies at Diagenode

When asked about how they choose their antibody of interest, researchers often point to their prior experiences with the antibody, comparative results, and well-cited products. However, researchers may not necessarily be satisfied with antibody performance. Oftentimes, they must optimize each and every ChIP assay with a suboptimal antibody simply to maintain "consistency" in future experiments even if better antibodies become available.

How did Diagenode address this quality problem?

Involvement in the academic network

Diagenode is a strong player in the growing epigenetic scientific community, collaborating with world-class research organizations such as, UC Davis, California, or Beijing Genomic Institute (BGI). Diagenode is a key partner in many active epigenetic consortiums such as **Blueprint** which aims to generate 100 reference epigenomes of hematopoietic cells from healthy individuals and from leukeaemic counterparts. In all of our collaborations, we are committed to use our state-of-the-art technologies to provide reference workflows and develop experimental and computational methods to improve detection, throughput, consistency, data analysis, storage, and retrieval.

Our dedication

Diagenode's selection of antibodies is exclusively dedicated for **epigenetic research**. We assign our R&D and production experts to continually deliver novel antibodies of the **highest quality**. The current collection targets histone modifications, chromatin associated proteins, modified bases of the DNA, transcription factors and CRISPR/Cas9 nuclease.

Strict quality standards

As our partners and clients require ALL information about an antibody to be available, we indicate **all specifications** on our technical data sheets. Only data generated internally or by official partners are considered acceptable. A bioinformatics QC pipeline is set up to 1) constantly control and objectively evaluate every step of the antibody production workflow and 2) facilitate improvements in our processes. We pay particular attention to the **batch-to-batch** variations and thus keep track of every batch in order to make them comparable.

Robust and standardized production

In our pursuit of achieving the highest quality, Diagenode only uses thoroughly tested and **validated methods** for antibody development and production. The standardized methods provide uniform conditions, which in turn guarantees **high product quality** without technical bias. We apply the same quality standards to our bioinformatics. The **robust QC pipeline** incorporates only well-established, properly tested informatics elements to analyze information from all possible sources to evaluate antibodies.

Innovation

Diagenode has developed innovative tools such as DNA and chromatin shearing equipments (**Bioruptor®**), automated devices (**IP-Star® automated workstation**), and **new innovative reagents** that we established as standards for Next-Generation Sequencing. We perform every ChIP-Seq step at Diagenode using the same tools as our customers. We constructed a powerful bioinformatics system that includes optimized software tools, from base calling through reference alignment to peak detection and extended tertiary analysis. That is a huge investment in time and material resources, but this enables us to select only the **best performing** candidates.

Expert technical support

The Diagenode marketing, sales, and technical teams consist of experts with actual epigenetics bench experience. Our team's expertise is unmatched as we provide quick and expert advice for the optimization of manual or automated epigenetic assays.

Antibodies are the backbone of immunoprecipitation assays

More than in any other immuoprecipitation assays, quality antibodies are critical tools in many epigenetics experiments. Since 10 years, Diagenode has developed the most stringent quality production available on the market for antibodies exclusively focused on epigenetic uses. All our antibodies have been qualified to work in epigenetic applications.

Immunoprecipitation

Immunoprecipitation has proven to be an invaluable investigational tool in epigenetics. It is routinely employed to explore the role of DNA-protein, RNA-protein or protein-protein interactions in the epigenome landscape and gene regulation.

Technically, it allows the determination of the relative abundance and stoichiometric distribution of a given target within a cell or a tissue. Basically, the success of an immunoprecipitation assay is dependent on two main factors, the abundance of antigen in the original sample, and the specificity and affinity of the antibody for its cognate antigen.

In epigenetics, three main targets are important for immunoprecipitation:

Chromatin

Chromatin Immunoprecipitation (ChIP) is used for studying chromatin structure and function. The antibodies used here are targeted against the epitopes of chromatin modifying proteins, histone deacetylase or demethylase enzymes, as well as histones and their various modifications.

DNA Methylation

DNA Immunoprecipitation is performed in order to study the role of DNA methylation in epigenetics and gene regulation. It targets mainly the 5-methylcytosine but also several variants like 5-hydroxymethylcytosine (5-hmC), 5-carboxylcytosine (5-caC) and 5-formylcytosine (5-fC).

Non-Coding RNA

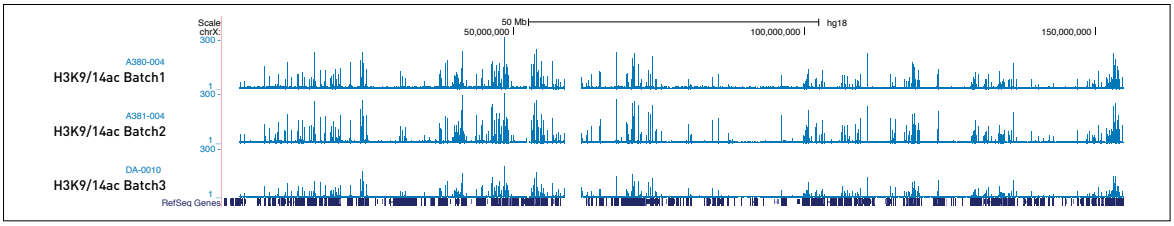
RNA Binding Protein Immunoprecipitation (RIP) is a pulldown experiment which helps to explain non-coding RNA mechanisms. RNAi proteins and complexes like the Argonaute family, the RISC complex and Dicer have been particularly valuable in improving the understanding of RNA and gene regulation.

Antibodies are targeted against RNA modifications (e.g. N6-meA) and non-coding RNA binding proteins.

Immunoprecipitation is divided into seven basic steps:

1. Fixation of the Chromatin (not in Native ChIP or in DNA methylation studies)
2. Sample preparation: Lysis of cells to release the antigen (fixed or non-fixed chromatin, or native DNA)
3. Formation of the antibody-antigen (immune) complex
4. Precipitation of the antibody-antigen complexes
5. Washing of the antibody-antigen complexes
6. Elution of the immune-enriched material and analysis (e.g. qPCR, Next-Generation Sequencing etc.)
7. Troubleshooting

Batch-to-batch comparison



DIAGENODE INNOVATING EPIGENETIC SOLUTIONS

Diagenode, a world leader in the field of epigenetics develops and commercializes innovative instruments and reagents. Founded in 2003, Diagenode is a global company with headquarters in Liege, Belgium and New Jersey, USA. Products can also be purchased through our extensive dealer network.

CURRENT PROMOTIONS

Visit our website to see the latest promotions and offers we have on Diagenode's products.https://www.diagenode.com/promotions

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You can also ask our customer support (custsupport@diagenode.com, custsupport.na@diagenode.com) to search our knowledge database to learn more about the technical aspects of our products and techniques, for information on independent reviews, or to find out how a product is used in a particular type of research.

ASK THE EXPERTS

TECHNICAL SUPPORT

Please, do not hesitate to contact our customer support team if you have any questions about the design of your ChIP-seq experiment or the bioinformatics data analysis.

Contact for Europe, Asia, Oceania and Africa:
custsupport@diagenode.com

Contact for North and South America:
custsupport.na@diagenode.com

DIAGENODE HEADQUARTERS

DIAGENODE S.A.
BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

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

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