

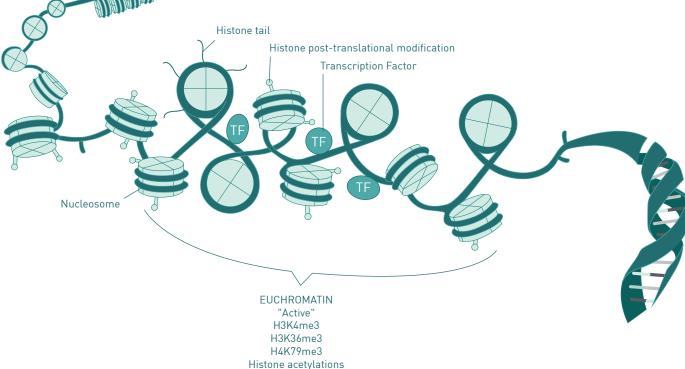


Chromosome H3K9me3 H3K27me3 H4K20me3 **HETEROCHROMATIN** "Silent" Histone tail

Diagenode helps you master Chromatin Analysis

Chromatin structure plays a key role in regulating gene expression by making DNA accessible to transcriptional machinery and transcription factors. The packaging of DNA into nucleosomes forms a closed structure that is not highly accessible to transcriptional elements whereas the open nucleosome structure allows DNA to be accessible.

Diagenode offers a number of solutions to help you analyze chromatin and the role of transcriptional machinery including ChIP kits, library preparation kits, ChIPmentation kits, CUT&Tag kit and pA-Tn5, antibodies and ATAC-seq kits.



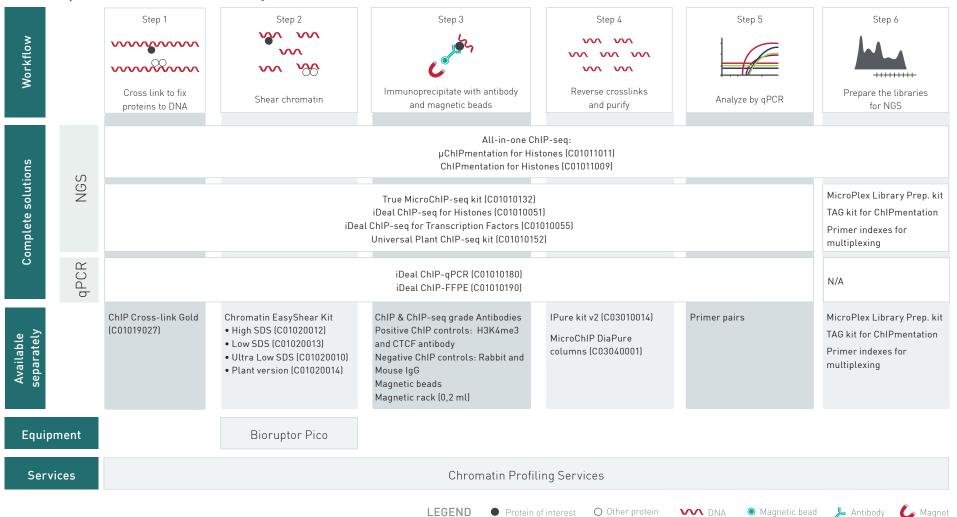
Innovating Epigenetics Solutions

I. Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) determines the location of DNA binding sites on the genome for a protein of interest, giving insights into gene expression regulation. ChIP involves the selective enrichment of a chromatin fraction containing a specific antigen. Antibodies that recognize a protein or protein modification are used to determine the relative abundance of that antigen at a specific locus or loci.

With an exclusive focus on epigenetics, Diagenode provides the highest quality epigenetics products on the market. Our complete suite of ChIP and ChIP-seq solutions includes extensively validated ChIP and ChIP-seq grade antibodies, chromatin shearing devices (Bioruptor®), kits for chromatin shearing, specialized kits for ChIP and ChIP-seq as well as for library preparation.

Optimal solutions for your end-to-end ChIP



I.I Chromatin preparation

A successful chromatin preparation relies on the optimization of cross-linking, cell lysis and sonication. Our **Chromatin EasyShear Kits** together with the **Bioruptor** ultrasonicator combine efficient cell lysis and chromatin shearing leading to consistent results.

Each Chromatin EasyShear Kit provides optimized reagents and a thoroughly validated protocol according to your specific experimental needs. SDS concentration is adapted to each workflow taking into account target-specific requirements.

- ✓ Obtain perfect fragment size for high quality ChIP Magnetic beads for more reproducible results
- Keep the epitopes accessible to the antibody
- Get consistent results from different samples and runs
- ✓ Benefit from the optimal kit for your specific experimental needs

		KITS				
		Chromatin EasyShear Kit Ultra Low SDS	Chromatin EasyShear Kit <i>Low SDS</i>	Chromatin EasyShear Kit for Plant	Chromatin EasyShear Kit <i>High SDS</i>	
	Cat. No.	C01020010	C01020013	C01020014	C01020012	
	Sample type	Cells, tissue	Cells, tissue	Plant tissue	Cells Histones Low	
	Target	Histones	TF	Histones		
FEATURES	Amount of starting material	Standard	Standard	Low and standard		
ATU	Nuclei isolation	Yes	Yes	Yes	No	
田	SDS concentration	< 0.1%	0.2%	0.5%	1%	
	Corresponding to shearing buffers from	iDeal ChIP-seq Kit for Histones ChIPmentation Kit for	iDeal ChIP-seq Kit for Transcription Factors iDeal ChIP a DCR Kit	• Universal Plant ChIP-seq Kit	• True MicroChIP-seq Kit • µChIPmentation for Histones	
		Histones	iDeal ChIP-qPCR KitiDeal ChIP-FFPE Kit		пізійнеѕ	

The shearing device of choice for chromatin preparation for ChIP

The **Bioruptor** is the latest innovation in shearing and represents a new breakthrough as all-in-one shearing system that delivers optimal and reproducible chromatin shearing while preserving high protein integrity.

Visit our website to learn more about our different models of Bioruptor and their features.

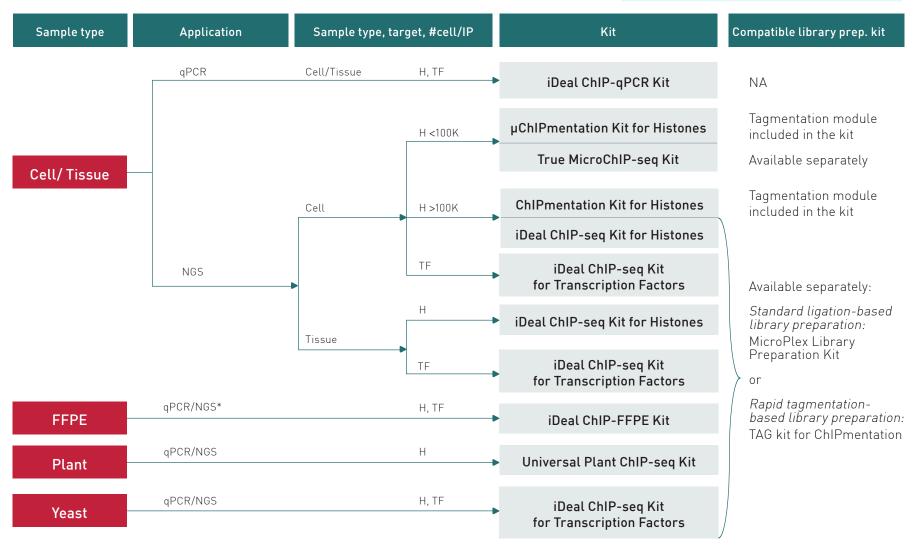


I.II Chromatin immunoprecipitation kits

Diagenode's optimized, high performance ChIP kits are the result of over 15 years of research and development. We provide a flexible platform that addresses specific research needs with a vast array of optimized ChIP kits. In addition, we provide the industry's most validated ChIP and ChIP-seg antibodies using a large variety of validation methods.

- Optimized robust protocols
- Magnetic beads for more reproducible results
- ✓ Perfect match of sequencing results with reference dataset
- ✓ Many species tested (human, mouse, rat, horse, chicken, cow, pig, plants and more)

Which ChIP kit is right?



I.III Library preparation kits

Diagenode's library preparation kits have been extensively validated with ChIP-seq samples. The MicroPlex Library Preparation Kit, which uses a simple 3-step protocol, is an optimal choice for library preparation, especially for very low inputs of DNA down to 50 pg. Moreover, the kits are available with either single or dual index options.

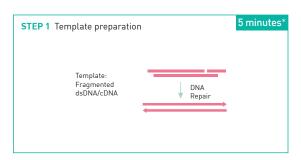
MicroPlex Library Preparation Kit - Features					
Sample	Fragmented dsDNA				
Input	50 pg - 50 ng				
Protocol	3 steps in one tube				
Hands on time	2 hours				
Intermediate purification	no				
Sequencing technology	Illumina®				
Indexes	Single	Dual, Unique Dual			
Multiplexing	Up to 12 samples	Up to 384 samples			
Indexes	Included in the kit	Available separately			
Manual protocol	✓	✓			
Automated protocol	✓	✓			

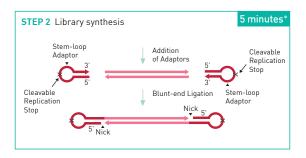
Available products					
MicroPlex Library Preparation Kit v3 - with dual indexes					
KITS*:					
C05010001 - MicroPlex Library Preparation Kit v3 /48 rxns					
UNIQUE DUAL INDEXES: C05010008 - 24 UDI for MicroPlex v3 - Set I /48 rxns C05010009 - 24 UDI for MicroPlex v3 - Set II /48 rxns					
DUAL INDEXES: C05010003 - 24 Dual indexes for MicroPlex Kit v3 /48 rxns C05010004 - 96 Dual indexes for MicroPlex Kit v3 - Set I /96 rxns C05010005 - 96 Dual indexes for MicroPlex Kit v3 - Set II /96 rxns C05010006 - 96 Dual indexes for MicroPlex Kit v3 - Set III /96 rxns C05010007 - 96 Dual indexes for MicroPlex Kit v3 - Set IV /96 rxns					
*The indexes for multiplexing are not included in the kits.					

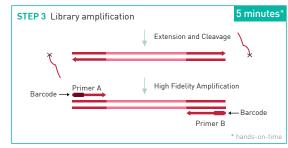
MicroPlex Library Preparation Kit v2 - with single indexes

C05010012 - MicroPlex Library Preparation Kit v2 (12 indexes) /12 rxns

How the MicroPlex Library Preparation Kit Works







Microplex workflow - protocol with dual indexes.

An input of 50 pg to 50 ng of fragmented dsDNA is converted into sequencing-ready libraries for Illumina® NGS platforms using a fast and simple 3-step protocol.

A quick glance: ChIP kit features

*H: Histones

*TF: Transcription Factor

Sagarolia 💹 🖳		KIT							
		µChIPmentation for Histones	ChIPmentation for Histones	iDeal ChIP-qPCR	iDeal ChIP-seq for Histones	iDeal ChIP-seq for TF	True MicroChIP- seq	iDeal ChIP-FFPE Kit	Universal Plant ChIP-seq
FEATURES	Downstream application	NGS	NGS	qPCR	qPCR, NGS	qPCR, NGS	qPCR, NGS	qPCR, NGS	qPCR, NGS
	Starting material: cells per 1 chromatin prep	No. of IP x No. of cells	700 K - 7 M	H*: 700 K - 7 M TF*: 25 M	700 K - 7 M	25 M	No. of IP x No. of cells	-	-
	Number of cells per 1 IP	10 K - 100 K	5 K - 1 M	H: 100 K - 1 M TF: 4 M	100 K - 1 M	4 M	10 K - 100 K	-	-
	Starting material: tissue per 1 chromatin prep	-	-	H: 20 mg - 30 mg TF: 200 mg	20 mg - 30 mg	200 mg	-	up to 6 slides	0.1 - 2 g
	Amount of tissue per 1 IP	-	-	H: 1.5 - 5 mg TF: 30 mg	1.5 - 5 mg	30 mg	-	up to 6 slides	0.007 - 0.13 g
	Target	Histones	Histones	Histones, TF	Histones	TF	Histones	Histones, TF	Histones
	Included buffers	Cell lysis, Chromatin shearing, Immunoprecipitation							
	DNA purification	n/a	n/a	✓	✓	✓	✓	✓	✓
	Control antibodies	IgG, H3K4me3	IgG, H3K4me3	-	IgG, H3K4me3	IgG, CTCF	IgG, H3K4me3	-	IgG, H3K4me3
	Control primers	(+)GAPDH TSS, (-) Myoglobin exon 2	Human: (+)GAPDH, (-)Myoglobin exon 2	-	(+)GAPDH TSS, (-)Myoglobin exon 2	Human: (+)H19, (-)Myoglobin exon 2	(+)GAPDH TSS, (-)Myoglobin exon 2	-	Arabidopsis: (+)FLC- ATG, (-)FLC-Intron1
	Library prep reagents	✓	✓	n/a	Separately in MicroPlex Library Preparation Kit or TAG kit for ChIPmentation				
	Indexes	Available separately	Available separately	n/a		ļ	Available separately		
ORDERING	Manual Kits	C01011012 (8 rxns) C01011011 (24 rxns)	C01011009 (24 rxns)	C01010180 (24 rxns)	C01010050 (10 rxns) C01010051 (24 rxns) C01010059 (100 rxns)	C01010054 (10 rxns) C01010055 (24 rxns) C01010170 (100 rxns)	C01010132 (20 rxns)	C01010190 (24 rxns)	C01010152 (24 rxns)
	Automated Kits - for IP-Star	-	C01011009 (24 rxns)	C01010181 (24 rxns)	C01010057 (24 rxns) C01010171 (100 rxns)	C01010058 (24 rxns) C01010172 (100 rxns)	C01010140 (16 rxns)	-	C01010153 (24 rxns)
ORI	Compatible Chromatin EasyShear Kit	C01020012	C01020010	C01020013	C01020010	C01020013	C01020012	C01020013	C01020014
	Services	✓	✓	✓	✓	✓	✓	✓	✓

This table presents the standard quantity of cells/tissues for each kit. It is possible to scale up or scale down the quantity of the material - please refer to the corresponding manual.

II. Tagmentation-based technologies

Next generation sequencing technology in the last decade has allowed higher sample throughput at a significantly reduced cost, necessitating the development of novel sequencing applications and methods. In one such method, tagmentation, a modified, hyperactive transposase (Tn5) cuts double-stranded DNA and simultaneously ligates linker sequences to both ends in short reaction time.

The high efficiency of this approach reduces both the input required as well as hands-on time compared to ligation-based library preparation methods.

Tagmentation offers numerous benefits:

- ✓ Supports new barcoding approaches
- Compatible with many different library preparation methods such as ATACseq, CUT&TAG, ChIL, ChIPmentation, TIP-seq, CHANGE-seq and others
- Comprehensive with supporting reagents as well as optimized and complete tagmentation-based solutions complement our Tn5

Products for tagmentation

- Tagmentase (Tn5 transposase) unloaded (C01070010) not loaded with DNA oligos, can be loaded with custom DNA oligos
- Tagmentation Dilution Buffer (C01070011) for dilution of Diagenode Tagmentase before or after transposome assembly
- Tagmentase (Tn5 transposase) loaded (C01070012) preloaded with Nextera sequencing adaptors compatible with Illumina sequencing platforms
- **Tagmentation Buffers** should be used in combination with Diagenode Tagmentase (Tn5 transposase) for any tagmentation reaction. Two versions available:
 - (1x) (C01019042) for low volume samples, it does not require any dilution. Application example: ChIPmentation
 - (2x) (C01019043) used as half of the total volume reaction. Application example: ATAC-seq
- **Primer indexes for Tagmented libraries** libraries multiplexing up to 72 samples:
 - 24 Single indexes for tagmented libraries (C01011032)
 - 24 UDI for tagmented libraries Set I (C01011034)
 - 24 UDI for tagmented libraries Set II (C01011036)
 - 24 UDI for tagmented libraries Set III (C01011037)

II.I ATAC-seq

ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) allows for assessing genome-wide chromatin accessibility. The technology is based on the use of the transposase Tn5 which cuts exposed open chromatin and simultaneously incorporates adapters for subsequent amplification and sequencing.

- Assess open regions of chromatin at single nucleotide resolution
- Gain insight into gene regulation and understand open chromatin signatures
- Determine nucleosome positions at single nucleotide resolution

- Optimized protocol for cells as little as 50K per tagmentation reaction
- ✓ Highly efficient nuclei extraction from tissue
- Optimized library prep step to avoid overamplification
- ✓ Easy and efficient DNA capture after the tagmentation reaction using Diagenode's DiaPure columns
- Robust protocol with high reproducibility between replicates and repetitive experiments

Optimized solutions for ATAC-seq:

- For cells: ATAC-seq kit (C01080002) + primer indexes for tagmented libraries
- For tissue: ATAC-seq package for tissue (C01080006) complete solution including all reagents

Primer indexes for tagmented libraries

- 24 Single indexes for tagmented libraries (C01011032)
- 24 UDI for tagmented libraries Set I (C01011034)
- 24 UDI for tagmented libraries Set II (C01011036)
- 24 UDI for tagmented libraries Set III (C01011037)

Separately available products:

- Tagmentase (Tn5 transposase) loaded (C01070012)
- Tagmentase (Tn5 transposase) unloaded (C01070010)
- Tagmentation Buffer (2x) (C01019043)
- Tissue Nuclei Extraction for ATAC-seg (C01080004)
- MicroChIP DiaPure columns (C03040001)

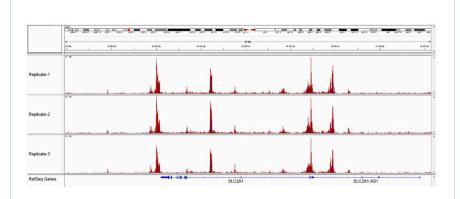
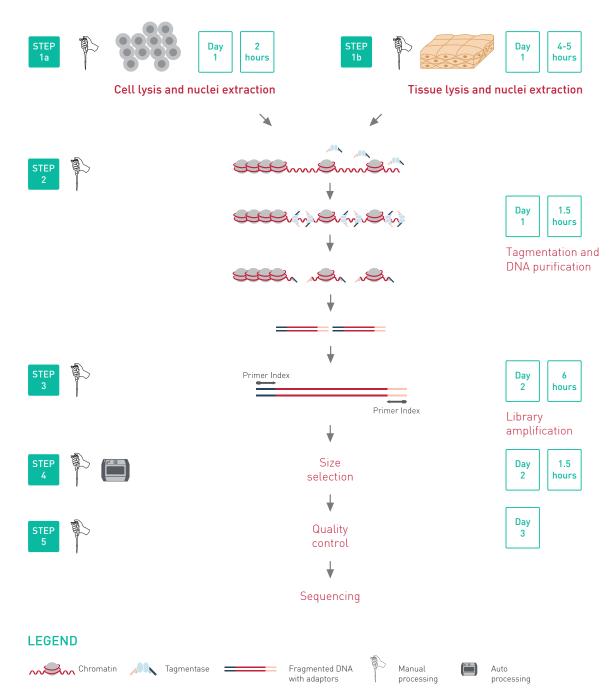


Figure 1. Sequencing profiles of ATAC-seq library (3 replicates) prepared with the Diagenode ATAC-seq kit and 24 UDI for tagmented libraries (Cat. No. C01011034) on 50,000 nuclei from K562 cells.

ATAC-seq Workflow



The ATAC-seq protocol consists of 3 steps: nuclei preparation, tagmentation and library amplification. First, the cells undergo the lysis, ending with the crude nuclei. Then, the nuclei are incubated with a Tagmentase (Tn5 transposase), which cuts the genomic regions associated with open chromatin and inserts the sequencing adaptors.

Finally, the generated libraries are amplified and can be used for sequencing. High-throughput sequencing will then detect peaks, in open regions of the chromatin only, giving a map of the chromatin status in the whole genome of the sample.

II.II ChIPmentation

Diagenode's unique ChIPmentation technology, based on **tagmentation**, enables the integration of library preparation during ChIP itself using a transposase loaded with sequencing adaptors. Unlike standard library preparation techniques that require many steps, ChIPmentation incorporates an easier and shorter protocol. Moreover, this reduced number of steps allows successful IPs on lower amounts of chromatin, which makes it ideal to analyze numerous histone marks on each chromatin sample.

- Easier and faster than classical ChIP-seq
- ✓ One step library preparation
- Elimination of sequencing adapter dimers
- High quality sequencing data
- Solution of choice for high throughput projects

Optimized solutions for:

- Low cell number μChIPmentation for Histones (C01011011) for as little as 10,000 cells per reaction, optimized protocol for FACS sorted cells
- Standard cell number ChIPmentation Kit for Histones (C01011009)
- Library preparation using tagmentation TAG Kit for ChIPmentation (C01011030) can be used with any ChIP protocol
- Multiplexing Primer indexes for tagmented libraries: 24
 UDI for tagmented libraries, Set I III (C0101134, C01011036, C01011037)

Separately available products:

- Standalone Tn5 transposase: Tagmentase loaded (C01070012)
 and Tagmentase unloaded (C01070010)
- Tagmentation Buffer (1x) (C01019042)
- ChIP-seq grade antibodies

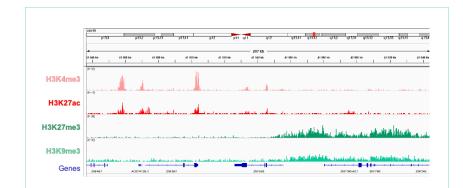
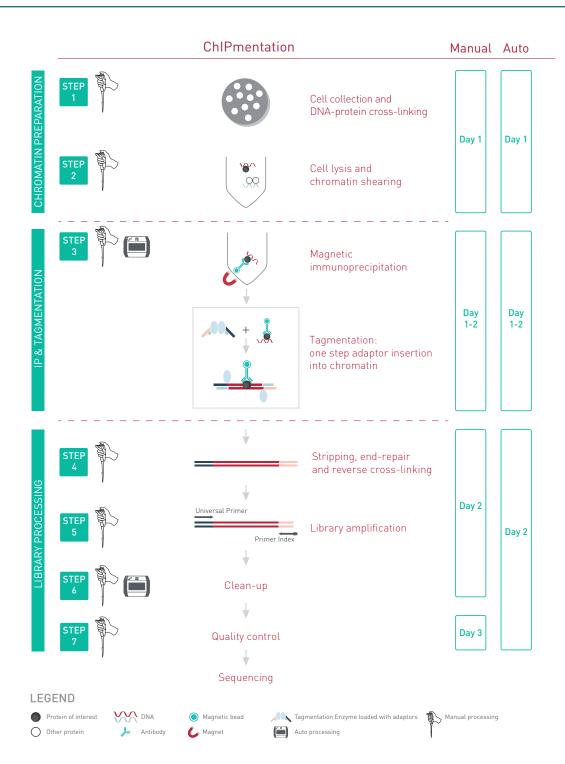


Figure 2. Sequencing profiles of µChIPmentation libraries

Chromatin preparation and immunoprecipitation have been performed on 10,000 cells using the μ ChIPmentation Kit for Histones (Cat. No. C01011011) and 24 SI for Tagmented libraries (Cat. No. C010111032) using K562 cells. The Diagenode antibodies targeting H3K4me3 (Cat. No. C15410003), H3K27ac (Cat. No. C15410196), H3K27me3 (Cat. No. C15410195) and H3K9me3 (Cat. No. C15410193) have been used.

ChIPmentation Workflow



ChIPmentation: after cell collection, the DNA and associated proteins are crosslinked in living cells using formaldehyde. Then cells are lysed and chromatin is sheared by sonication (Bioruptor) to obtain the fragments between 100 - 600 bp. Crosslinked protein-chromatin complexes of interest are selectively immunoprecipitated from the cell debris using an appropriate protein-specific antibody. Tagmentase loaded with sequencing adaptors is added enabling one-step library preparation: the tagmentase introduces sequencing-compatible adaptors in a single-step reaction directly on beadbound chromatin.

After stripping, end-repair and reverse cross-linking, the generated libraries can be amplified and used for sequencing.

II.III CUT&Tag

CUT&Tag-sequencing (Cleavage Under Targets and Tagmentation) is a new chromatin profiling method providing high quality sequencing data from low starting amount. This alternative to ChIP-seq method, combines antibody-targeted controlled cleavage by a protein A-Tn5 fusion with massively parallel DNA sequencing to identify the binding sites of DNA-associated proteins. Diagenode's iDeal CUT&Tag kit for Histones provides an optimized protocol for a rapid chromatin profiling on histone marks. The protocol is optimized for native cells (10,000-300,000 cells per reaction) and can be completed within 2.5 days.

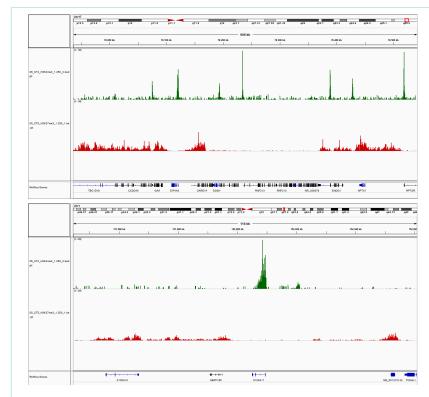


Figure 3. CUT&Tag profiles show typical enrichments specific for a given histone mark. Representative screenshots for H3K27me3 (red) and H3K4me3 (green) are shown at selected loci. H3K27me3, which marks inactive regions (red), does not show enrichment over open chromatin regions, marked by H3K4me3 (green). CUT&Tag was performed using 50,000 of K562 cells and Diagenode H3K4me3 polyclonal ChIP-seq antibody (Cat. No. C15410003), and H3K27me3 polyclonal ChIP-seq grade (Cat. No. C15410195).

For a complete CUT&Tag protocol the following items must be purchased:

- iDeal CUT&Tag for Histones C01070020 including all reagents for CUT&Tag workflow (buffers, pA-Tn5, CoA beads, DNA purification)
- Antibody package for CUT&Tag (anti-rabbit C01070022 or antimouse C01070023) - including the secondary antibody, positive and negative control antibodies and primers
- Antibody recognizing the protein of interest choose one of Diagenode's validated antibodies
- Primer indexes for tagmented libraries for multiplexing up to 72 samples

Separately available products:

• Fusion protein – compatible with CUT&Tag, but also other applications like ACT-seq, CoBATCH, TIP-seq and more.

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pA/Tn5 Transposase - loaded (C01070001)
pA/Tn5 Transposase - unloaded (C01070002)
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- CUT&Tag grade antibodies
- DNA purification

IPure kit v2 (C03010014)

MicroChIP DiaPure columns (C03040001)

• Primer indexes for tagmented libraries

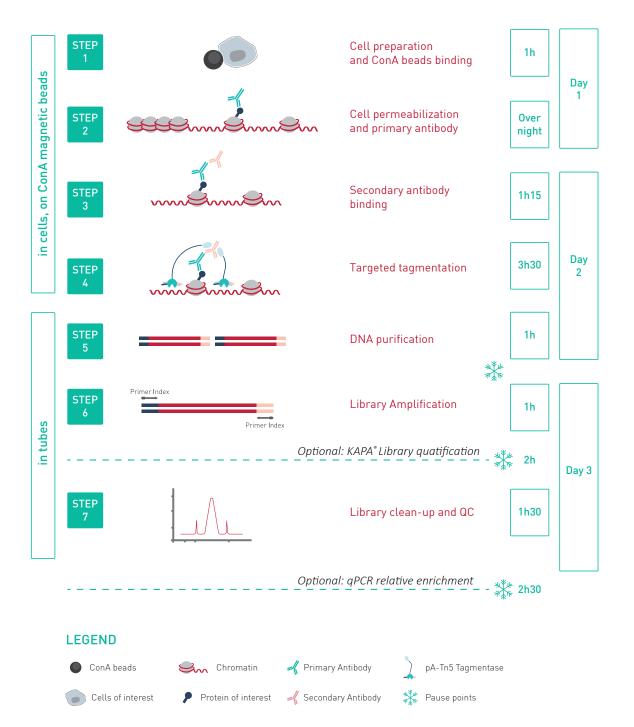
24 Single indexes for tagmented libraries (C01011032)

24 UDI for Tagmented libraries - Set I (C01011034)

24 UDI for Tagmented libraries - Set II (C01011036)

24 UDI for Tagmented libraries - Set III (C01011037)

CUT&Tag Workflow



The iDeal CUT&Tag protocol involves the binding of cells on solid phase ConA magnetic beads, allowing magnetic handling of the cells for the major steps of the protocol. Bead-bound cells are permeabilized and incubated with primary antibody against a target of interest and secondary antibody.

Then, Diagenode's protein pA-Tn5 Transposase -loaded is bound to the complex. Protein A guides Tn5 transposase on chromatin to the antibody attached to its target. Tn5 transposase is activated by Mg+2 ions to insert the sequencing adaptors into genomic regions of interest. DNA is then purified and the tagmented genomic regions of interest are amplified by PCR using Diagenode's Primer Indexes for tagmented libraries.

- Rapid and easy chromatin profiling assay for histones marks
 - Native cells: no fixation or chromatin fragmentation
 - Easy sample handling with ConA magnetic beads
 - Integrated library prep
- ✓ High resolution and sensitivity
- ✓ Low cell number required (10K – 300K)
- Lower sequencing depth and no input required compared to ChIP

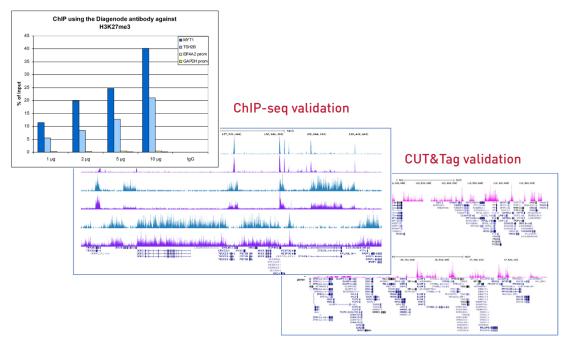
III. Antibodies

Obtaining highly sensitive and highly specific antibodies is challenging for researchers. Many antibodies that are used in experimental assays have limited validation behind them, and the data given by the provider are not always lot-specific.

Quality matters! That is why we validate the antibodies at Diagenode.

At Diagenode we assign our R&D and production experts to continuously deliver novel antibodies of the highest quality. Diagenode's selection is exclusively dedicated for epigenetics research. We validate our antibodies in epigenetic applications, including **ChIP**, **ChIP-seq** and **CUT&Tag**. Only the antibodies which pass the rigorous QC are released as **ChIP**, ChIP-seq or CUT&Tag grade. We validate each lot and we are transparent – batch-specific validation data are available on the website. We provide the antibodies you can trust! Check out the complete list of antibodies on our website www.diagenode.com

ChIP validation



DIAGENODE OFFERS A WIDE BREADTH OF ANTIBODIES FOR:

- Native and modified histones
- DNA methylation
- Chromatin modifying proteins
- Transcription factors
- RNA modifications
- Nuclear receptors
- CRISPR Cas9

Why Diagenode's antibodies?



UNMATCHED EXPERTISE

with 15+ years of epigenetics-focused antibody validation



BATCH-SPECIFIC DATA

available on the website



STRICT QUALITY STANDARDS

with rigorous QC lot-to-lot comparison



NUMEROUS VALIDATION STEPS

to guarantee results (includes peptide array confirmation, siRNA validation, ChIP, ChIP-seq, CUT&Tag)

Kits and reagents highlights

CHIP ON FFPE SAMPLES

iDeal ChIP-FFPE Kit

- ✓ Fast and user-friendly deparaffinization workflow
- ✓ Deparaffinization: less toxic than a conventional xylene-based method
- Easy recovery of re-hydrated tissue due to DiaFilter columns
- ✓ Fast chromatin preparation without enzymatic digestion
- ✓ A mild de-crosslinking preserves dsDNA
- ✓ Eluted DNA suitable for qPCR analysis or other down-stream applications



CHIP ON PLANT

Universal Plant ChIP-seq kit

- ✓ Specifically optimized for extracting plant chromatin
- ✓ Optimized crosslinking (crosslinking bags)
- ✓ Compatible with low input
- ✓ Higher enrichment
- ✓ Higher DNA recovery after ChIP
- ✓ Tested on many plant species (tomato, populus, Arabidposis, rice and others)
- ✓ Validated with MicroPlex Library Preparation Kit for library prep



DNA PURIFICATION

- I. The IPure kit v2 (C03010014 (24 rxns), C03010015 (100 rxns)), based on magnetic beads, is specifically optimized for efficient DNA purification after ChIP.
 - ✓ Significantly greater yields than with column-based purification
 - ✓ Recovery of small amounts of DNA
 - ✓ Straightforward protocol using magnetic beads
 - ✓ Toxic reagents not used (e.g. phenol/chloroform)
 - ✓ Provides pure DNA for any downstream application (e. g. NGS)

II. The MicroChIP DiaPure columns C03040001, is specifically optimized for efficient DNA purification of low concentrated samples.

- ✓ Perfect for low concentrated samples (elution from 6 μl)
- ✓ DNA recovery 70-90% (50bp 10kB)
- ✓ No toxic reagents (e.g. phenol/chloroform)
- ✓ Provides pure DNA for any downstream application (e. g. NGS)

Other solutions to use with our products or your own protocols

FIXATION REAGENT FOR INDIRECT PROTEINS

For proteins not bound directly to DNA, use **ChIP Cross-link Gold** (Cat. No. C01019027) for efficient protein-protein fixation in higher order or dynamic interactions.

CHIP NEGATIVE CONTROLS

Depending on the antibody of interest, choose between rabbit, mouse or rat IgG.

BEADS

Extensively validated magnetic and agarose beads validated for isolation of immune complexes in ChIP experiments.

MAGNETIC RACK

Use Diagenode's DiaMag 0.2 ml, for fast and efficient isolation of magnetic beads.

PRIMER PAIRS

Check out our list of ChIP/ChIP-seg grade primer pairs on our website.



www.diagenode.com