Chromatrap

A more efficient, sensitive and robust method of chromatin immunoprecipitation (ChIP)

ADVANCEMENTS IN EPIGENETICS

Chromatrap® is a more efficient, sensitive and robust method of ChIP

Chromatrap® is a solid-state filter-based technology that significantly enhances and accelerates the important epigenetic research tool of chromatin immunoprecipitation (ChIP). It is rapidly developing into many areas of genome research and is now available for classical qPCR, sequencing and ChIP from formaldehyde fixed paraffin embedded samples (FFPE) using either spin columns or 96-well filter plates. The simplicity and efficacy of Chromatrap® ChIP assays, enabling more IPs per sample, using less starting material and more quickly than traditional assays which has made them a firm favourite with leading genetics research laboratories worldwide.

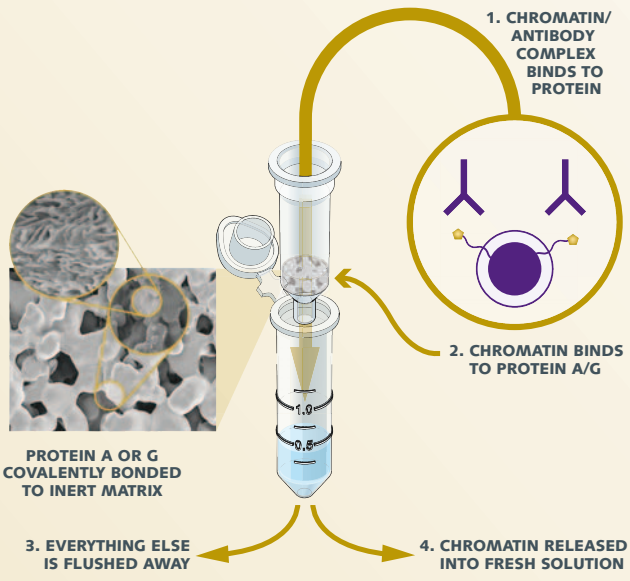
Compared to standard methods, Chromatrap® is:

- **Faster**
- **Easier**
- **More sensitive**
- **Less prone to errors**

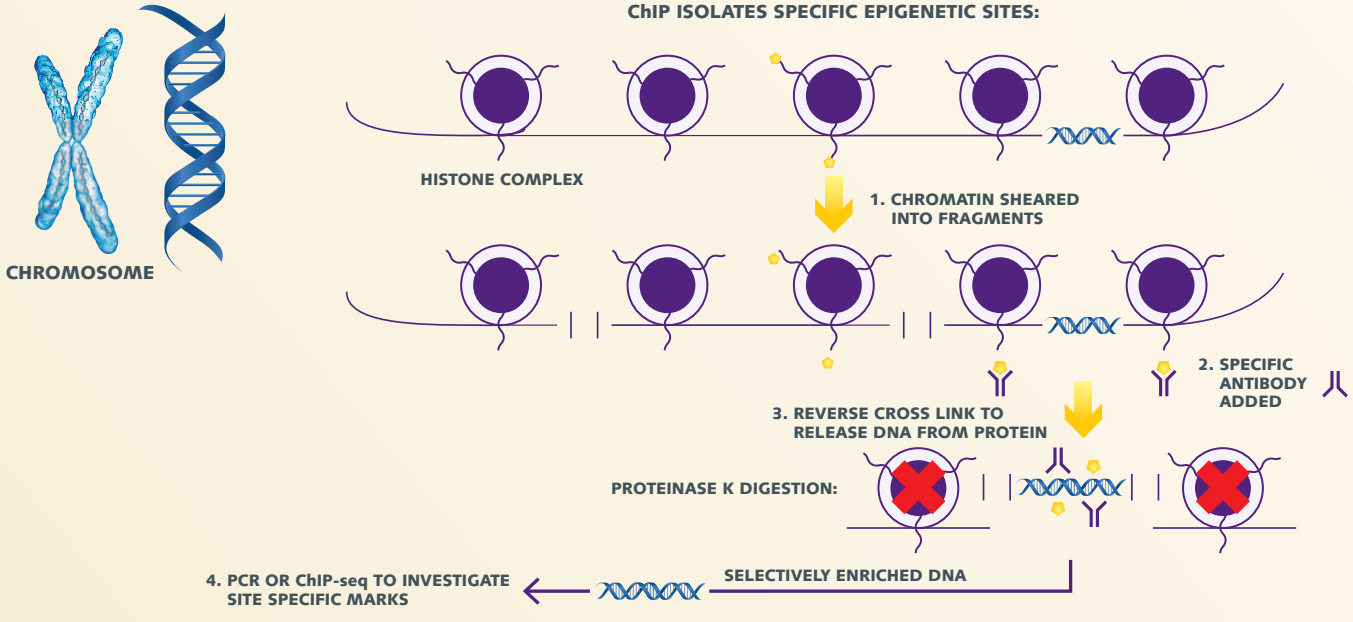
Chromatrap® kits use revolutionary spin columns or microplates which contain discs of an inert, porous polymer to which protein A or G has been covalently attached. This patented format is unique. During an assay, the chromatin/antibody complex is selectively retained by the disc. Flushing with three buffers and an elution step are all that is required to obtain the selectively enriched DNA, making Chromatrap® more efficient in the laboratory.

With under five hours from chromatin loading to qPCR-ready DNA, Chromatrap® significantly reduces the assay time for ChIP, enabling more samples to be analysed, increasing laboratory throughput and efficiency. Now available for enzymatic or sonicator-based DNA shearing and with or without control antibodies, the range of Chromatrap® ChIP products continues to expand. Further exciting new developments in the Chromatrap® range are planned, so do keep up to date with our website at: **www.chromatrap.com**

Chromatrap® offers an inert solid-phase scaffold for better immunoprecipitation



- Flow through solid phase scaffold
  - Inert material
  - Open structure
  - Pro A/G bound to surface
  - Promotes molecular movement
  - Better sample mixing
  - Minimises non-specific binding



Chromatrap® qPCR

Chromatrap® ChIP kits for qPCR rapidly improve ChIP using their unique patented technology streamlining the process, ensuring chromatin preparation to qPCR analysis can be performed in under 5 hours. The ChIP qPCR assay is a short convenient assay for low chromatin loading with very small slurry volumes (40 uls) with no DNA clean up steps prior to qPCR.

By avoiding the use of magnetic or agarose beads, non-specific background is eliminated together with reduced manual handling errors and subsequent sample loss.

Optimised elution buffer chemistry allows samples to be analysed directly in qPCR without the need for DNA clean up, further reducing sample loss.

The kits have also been created for 1000 ng sample sizes, which allows for better results from smaller sample sizes and greater flexibility as more IP assays can be performed in one sample.

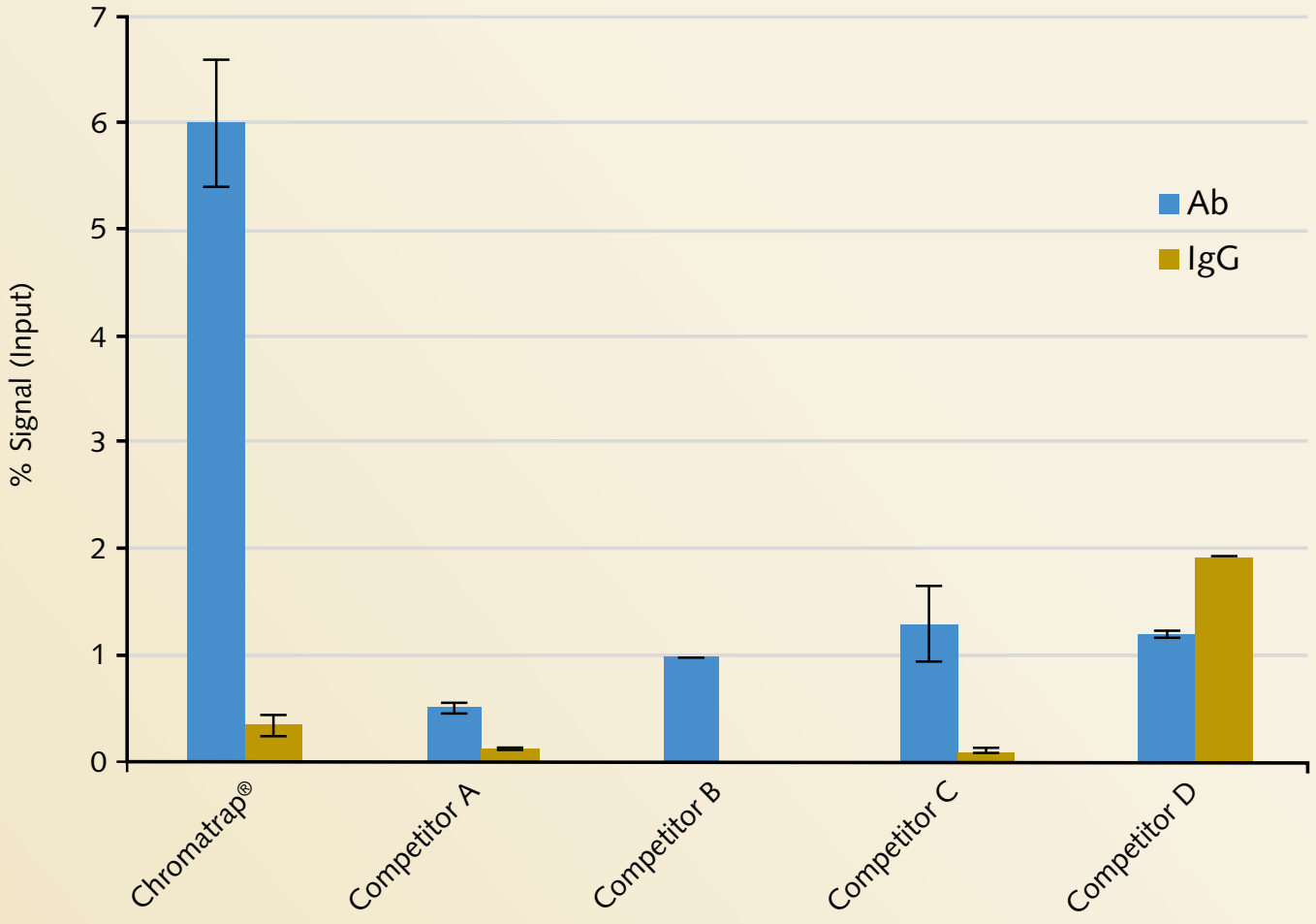
- *Designed for downstream process only*
- *Suitable for low chromatin loadings (1 µg-10 µg)*
- *Contains sufficient Protein A or Protein G based spin-columns, buffers and reagents to perform 24 chromatin immunoprecipitation*
- *Up to 10 chromatin preparations by sonication*
- *An enzymatic shearing format is also available*

Premium kits containing positive and negative antibody and primer sets to provide you with satisfaction of chromatin preparation.

Advantages:

- **ChIP in under 5 hours from chromatin preparation to qPCR**
- **Less manual handling, eliminating sample loss through multiple pipetting steps**
- **Single column and 96-well high throughput formats available**
- **Optimised for 1000 ng sample sizes: better results from smaller sample sizes for qPCR**
- **Allows more IP assays from a single sample**

Chromatrap® qPCR kits outperform all other chromatin immunoprecipitation methods



Chromatrap® ChIP-seq

Chromatrap® provides a simple and easy to use ChIP format, compatible with high and low cell numbers, validated on both transcription factor and epigenetic mark identification in primary and secondary cell lines. Compatible with direct, deep sequencing of enriched fragments, Chromatrap® ChIP-seq assays now enable unbiased, genome-wide understanding of protein-DNA regulatory networks. Excitingly, the inert solid-support matrix enables reproducible capture and genome-wide amplification of landmark regulatory complexes from low amounts of input chromatin.

Chromatrap® generates high quality reproducible data for challenging ChIP-seq experiments. From both high and low input chromatin, Chromatrap® allows genome wide transcription factor binding site and/or histone modification mapping, providing new insights into gene regulatory processes in normal physiology and disease.

The first challenge for any ChIP-seq experiment is to determine library quality and data validity. When analysed for quality control purposes, Chromatrap® ChIP-seq data meets the three ENCODE consortium recommendation levels; 1-FAST Q, 2-Coverage and 3-Peak and alignment thus providing consumer confidence when using ChIP validated antibodies. Chromatrap® derived FASTQ files, from high and low abundant, broad and narrow peak marks, always achieve FASTQC quantification above Q30 (Figure 1A). In addition, validation of the library preparation is observed with low duplication levels and high % of uniquely mapped reads. Chromatrap® routinely obtains very low levels of duplication <2% when looking for transcription factors and histone modifications within the human genome Figure 1B).

Using low and high chromatin loadings Chromatrap® achieve 95% uniquely mapped reads for low abundant transcription factors this is comparative to reads obtained with histone modifications.

QC validation	Chromatrap
FAST Q	>30
Coverage with 30 million reads	> 95% uniquely mapped reads for TF and histone modifications
Peak alignment	Significantly enriched peaks (MACS); Compatible for TF and histone modification mapping

Table 1. *Library quality control.*

Sample	Total reads	Uniquely mapped reads %	% duplication
EZH2 2 µg	35343366	32454438 (95.63)	0.91%
EZH2 30 µg	37931206	35542632 (96.00)	1.59%
H3k4me3	38383695	35819760 (95.72)	1.23%

Table 2. *Uniquely mapped reads for both narrow and broad peak marks.*

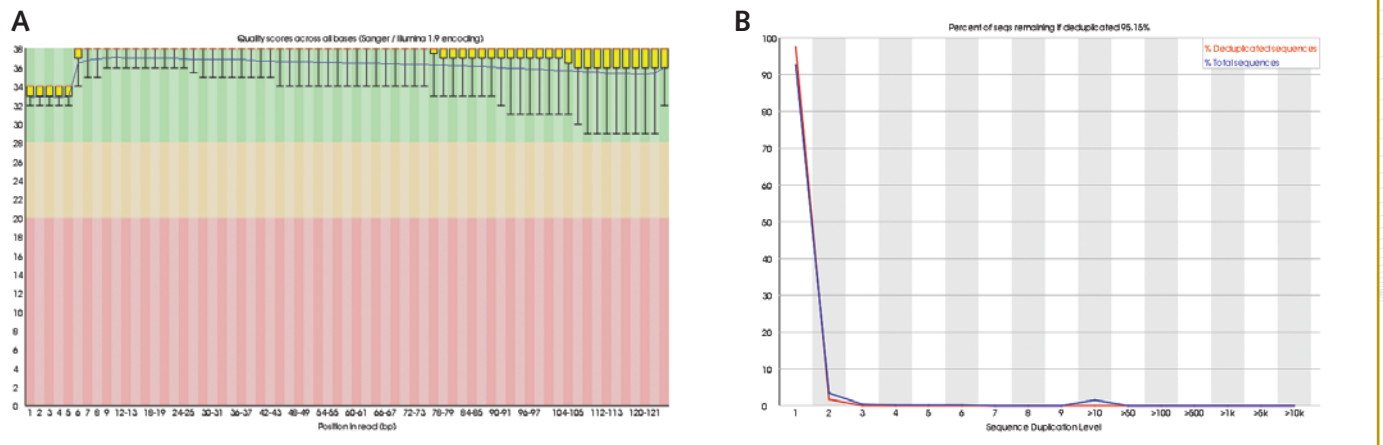


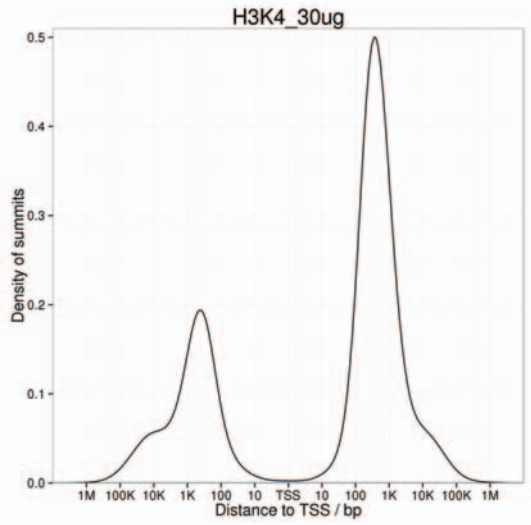
Figure 1. *High ratio of Q30 scores from FASTQC and low duplication levels in all Chromatrap® characterised libraries.*

Advantages of Chromatrap® ChIP-seq:

- Ample DNA to perform library prep from a single IP
- Positive target enrichment typically 15-20 fold when compared with background IgG control
- ChIP-seq from as little as 1-50 µg of chromatin
- Wide dynamic range allows lower chromatin loadings and minimises antibody requirements.
- Fully compatible with Illumina® MiSeq™ and HiSeq™
- Reduced IP incubation times

Chromatrap® ChIP-seq

When aligned to the reference genome (H19) and analysed for coverage, characteristic peak geometries are obtained as expected. H3k4me3 readout for example, routinely shows a characteristic distribution of signal in the context of the transcription start site, downstream coding and upstream regulatory regions (Figure 2).



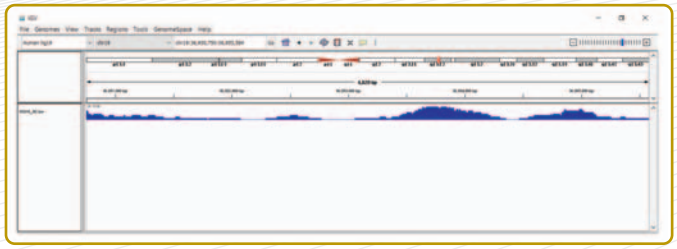


Figure 3 (above): *H3K4me3 ChIP-seq data set*
ChIP was performed using the Chromatrap® ChIP-seq kit (Cat. No. 500189) with 30 µg chromatin from Human Hec50 cells with 5 µg of antibody. ChIP DNA was sequenced on the Illumina® MiSeq and 30 million sequence tags were mapped to identify H3K4me3 (Catalogue 700010) binding sites. The image shows H3K4me3 bound to ZNF566 on chromosome 19.

Figure 2 (left): *H3K4me3 distribution*
The availability of Chromatrap® ChIP-seq in a 96-well plate format allows for high-throughput analysis and, for the first time, provides an unrivalled tool for simultaneously studying complex, co-ordinated gene regulation and epigenetic mechanisms on a global scale. The next level of sequencing analysis involves peak calling and alignment to the reference genome. Chromatrap® achieve robust peak calling for both high and low chromatin loadings.

Chromatrap® DNA Purification Plates

Chromatrap® provide 96-well high throughput plates for ultra-pure DNA purification. Using proprietary filtration media that offer much higher loadings of active material, assay times are under 5 minutes.

Buffers are optimised to remove any unwanted impurities while providing efficient DNA recovery from samples. The Chromatrap® DNA kit is designed for the purification and concentration of samples from PCR mixtures, ChIP and restriction enzyme digestions.

- **DNA samples ranging from 50 bp up to 23 kb can be purified with up to 98% recovery**
- **Up to 50 µg of DNA can be recovered efficiently and quickly using a Chromatrap DNA purification kit**

The Chromatrap® DNA 96 Micro Elution Purification Kit can purify up to 10 g DNA in small elution volumes (5-10 µl) providing a cleaner and more concentrated sample required for certain applications such as library preparation for DNA sequencing.

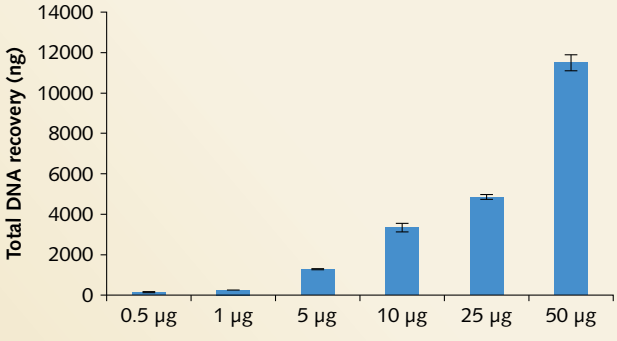


Figure 1. *Total DNA recovery (ng) of a range of DNA concentrations using the Chromatrap® 96 DNA Purification kit DNA clean up plate (0.5-50 µg).*

The power of microplate processing applied to ChIP assays

Chromatrap® 96 HT is a microplate-based system allowing for the first time up to **96 ChIP assays to be processed simultaneously in less than one working day**. This allows multiple antibody and gene targets to be investigated in parallel. The Chromatrap® 96 HT system can also be set up for automated liquid handling providing an even faster and more efficient method for performing ChIP assays.

Large, reliable data sets can be collected efficiently, enabling widespread effects to be studied across multiple samples and multiple cell types; all carried out simultaneously.

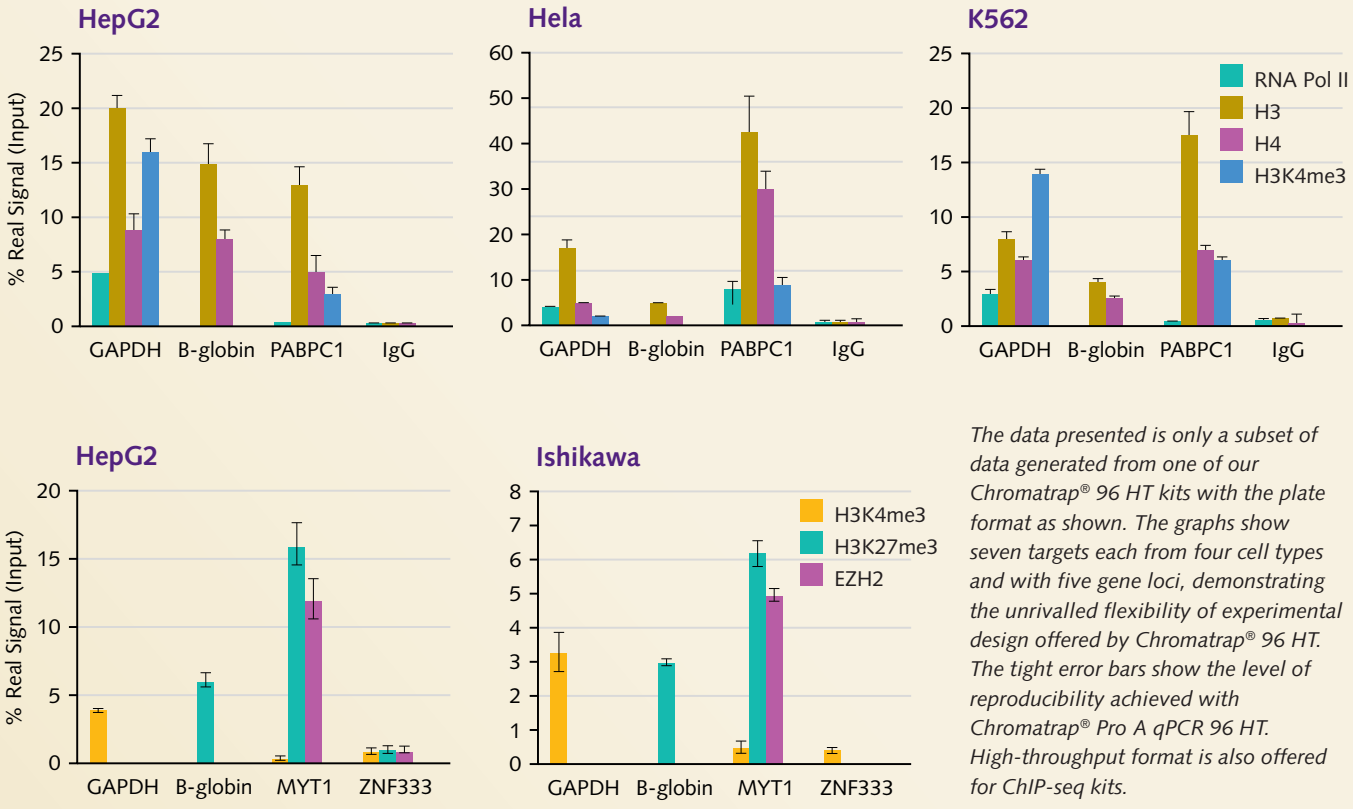
Example plate layout for 96 reactions

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

Key

Well	Cell Line	Antibody	Well	Cell Line	Antibody	Well	Cell Line	Antibody	Well	Cell Line	Antibody
1-3	HepG2	RNA Pol II	25-27	K562	H3K27me3	49-51	HeLa	HDAC	73-75	Ishikawa	IgG
4-6	HepG2	H3K4me3	28-30	K562	EZH2	52-54	HeLa	IgG	76-78	MCF7	RNA Pol II
7-9	HepG2	H3K27me3	31-33	K562	HDAC	55-57	Ishikawa	RNA Pol II	79-81	MCF7	H3K4me3
10-12	HepG2	EZH2	34-36	K562	IgG	58-60	Ishikawa	H3K4me3	82-84	MCF7	H3K27me3
13-15	HepG2	HDAC	37-39	HeLa	RNA Pol II	61-63	Ishikawa	H3K27me3	85-87	MCF7	EZH2
16-18	HepG2	IgG	40-42	HeLa	H3K4me3	64-66	Ishikawa	EZH2	88-90	MCF7	HDAC
19-21	K562	RNA Pol II	43-45	HeLa	H3K27me3	67-69	Ishikawa	ER-a	91-93	MCF7	ER-aa
22-24	K562	H3K4me3	46-48	HeLa	EZH2	70-72	Ishikawa	IgG	94-96	MCF7	IgG

7 targets, 4 cell types and 5 gene loci

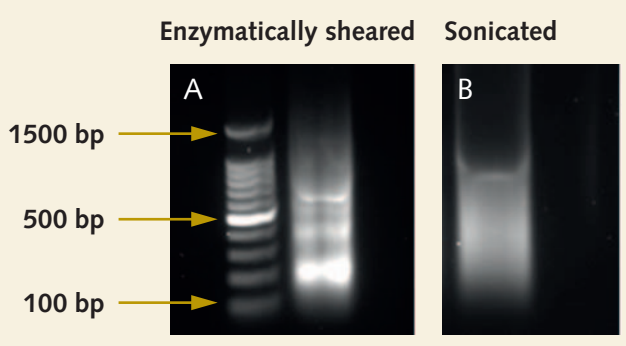


The data presented is only a subset of data generated from one of our Chromatrap® 96 HT kits with the plate format as shown. The graphs show seven targets each from four cell types and with five gene loci, demonstrating the unrivalled flexibility of experimental design offered by Chromatrap® 96 HT. The tight error bars show the level of reproducibility achieved with Chromatrap® Pro A qPCR 96 HT. High-throughput format is also offered for ChIP-seq kits.

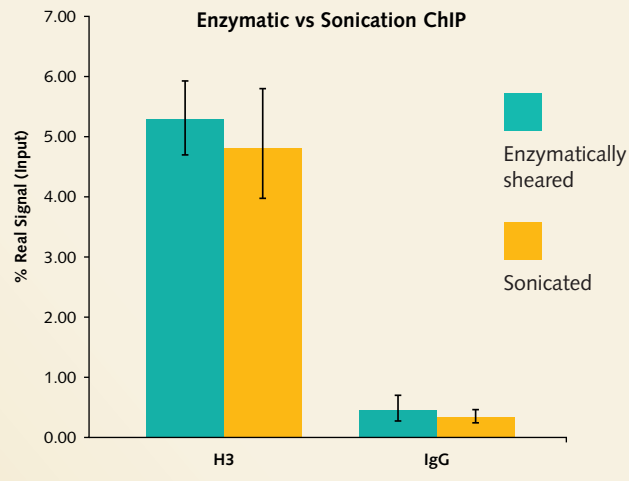
Chromatrap® enzymatic ChIP kits

Isolation of good quality, suitably fragmented chromatin is the most important prerequisite for a successful ChIP assay. Chromatin can be sheared enzymatically or by mechanical methods such as sonication. Enzymatic shearing can be advantageous where expensive sonication equipment is not available or where native chromatin is to be examined. The sheared chromatin is compatible with NGS sequencing library preparation kits.

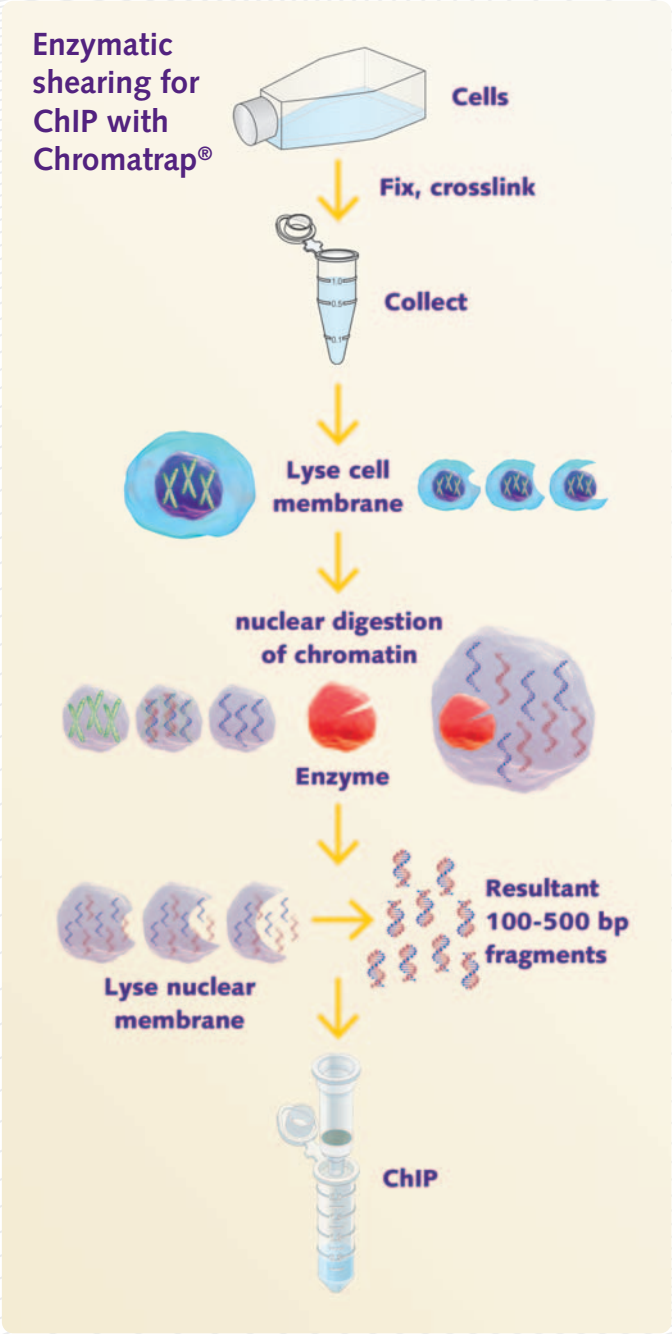
- No sonicator needed
- Compatible with sequencing
- All enzymes provided
- Ensures optimal shearing
- Enzymatic shearing kit available separately



Gel electrophoresis of Hec50 chromatin using the Chromatrap® Enzymatic Shearing Kit and Spin Column Sonication Kit. *The banding pattern, typical of enzymatic digestion, with fragments of 200 bp, 400 bp and 600 bp – ideal for ChIP using Chromatrap® (A). Uniform chromatin fragment lengths between 100 and 500 bp visualised with sonicated chromatin (B).*



H3 signal enrichment at the GAPDH promoter. *Excellent signal to noise ratio in both Hec50 chromatin prepared using the Chromatrap® Enzymatic Shearing Kit and the Spin Column Sonication Kit, strong signal was obtained at the GAPDH gene promoter following H3 IP.*



Chromatrap® Enzymatic ChIP Kits provide an excellent method for the preparation of high quality, ideally fragmented chromatin for ChIP analysis, in either spin column or filter plate format. These kits demonstrate excellent enrichment, independent of starting cell number. With their quick and simple protocol Chromatrap® Enzymatic ChIP Kits are the perfect cost-effective alternative to sonication.

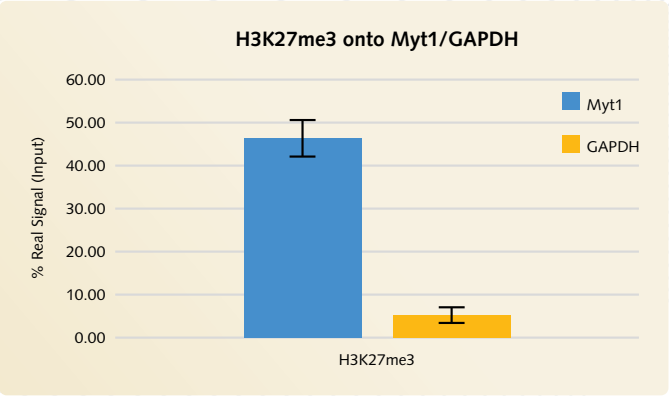
The optional Chromatrap® Enzymatic shearing kit supplies all reagents and buffers for up to 10 chromatin preparations, allowing you to determine optimal shearing conditions and generate enough chromatin to perform up to 24 ChIPs using a standard Chromatrap® ChIP spin column kit or up to 96 IPs using the Chromatrap® 96 HT microplate kit.

Chromatrap® Native ChIP

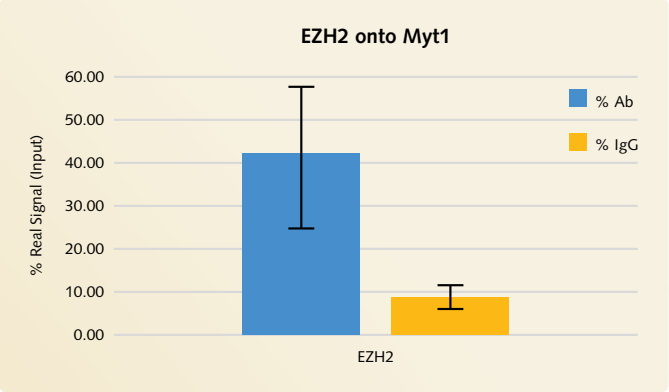
Chromatrap® Native ChIP kits provide an alternative to traditional cross-linked ChIP, which removes the need for fixing cells prior to extraction. In Native chromatin the proteins and histones wrapped around DNA are naturally linked and the traditional methods of shearing can easily disrupt the DNA-protein complexes. Therefore the DNA is biologically sheared using micrococcal enzyme digestion of the nuclei. This kit can be can be used for the study of histone modifications and certain abundant transcription factors that are likely to be bound to DNA.

Chromatrap® Native ChIP kits provide a quicker, easier and more efficient way of performing ChIP assays from native chromatin. Using the unique patented technology maximises the capture efficiency of the target chromatin/antibody complex. By avoiding the use of magnetic or agarose beads, non specific background is eliminated together with reduced manual handling errors and subsequent sample loss.

- **This kit is compatible for both qPCR and sequencing as a downstream process**
- **Wide dynamic range suitable for low and high chromatin loadings (1-50 µg)**
- **Better results from small sample sizes, thus more IP assays can be performed from just one sample**
- **The kit is ideal for the enrichment of histone modifications and some low abundant transcription factors**
- **Available in spin column and high throughput format**
- **No chemical fixation so cells remain in a more natural 'native' state**
- **Increased affinitiy of antibody bdinging to antigens on native chromatin as it is more accessible without chemically induced crosslinks**



Native ChIP: H3K27me3 onto Myt1/GAPDH: *High levels of enrichment are produced using the Chromatrap® Native ChIP kit onto the positive gene target MYT1. Specific pull down of H3K27me3 is demonstrated by a 10-fold difference between the positive gene target (MYT1) and negative gene target (GAPDH).*



Native ChIP: EZH2 onto Myt1: *Chromatrap® Native ChIP kit is sensitive enough to pull down low abundant transcription factors. Here, excellent signal to noise is demonstrated with the Chromatrap® Native ChIP kit when specific antibody enrichment (EZH2) is compared to the enrichment of non-specific IgG. This result is achieved with only 2 g of antibody is used per IP.*

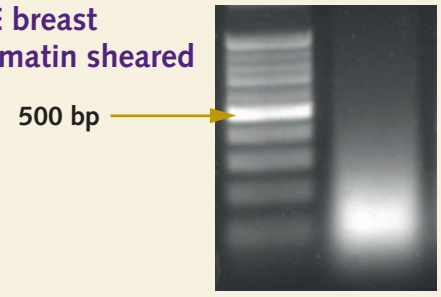
Native ChIP		Cross-linked ChIP	
ADVANTAGES	DISADVANTAGES	ADVANTAGES	DISADVANTAGES
Suitable for investigating histone marks and abundant targets.	Slightly longer due to overnight dialysis.	ChIP carried out in 5 hours!	Chemical fixation required.
No chemical fixation of cells; cells remain in a more natural 'native' state.	Can only shear chromatin enzymatically.	Can shear chromatin by either sonication or enzymatically.	Cannot capture chromatin from cells in their 'native' state.
In some cases there is increased affinity of antibody binding to antigens on native chromatin as it is more accessible.	Some low abundant transcription factors may not be detected.	Can investigate a wide range of histone marks and transcription factors.	Cross-linked chromatin can occasionally mask epitopes of some antibodies, affecting antibody/ chromatin binding.

Table 1 *Comparison of Chromatrap®'s native vs cross-linked ChIP kits*

Chromatrap® FFPE ChIP

FFPE-ChIP uses chromatin extracted from Formalin Fixed Paraffin Embedded (FFPE) tissue. FFPE is a standard method used to archive and preserve medical tissue biopsy samples. Due to the extensive cross-linking of proteins by formaldehyde, FFPE tissues present a particular challenge for ChIP analysis. In addition, the small size, delicate nature of the tissue and the difficulty in extracting samples from paraffin, can damage cellular complexes such as chromatin. ChIP assays from these samples have proved difficult and time consuming.

Human FFPE breast tumour chromatin sheared



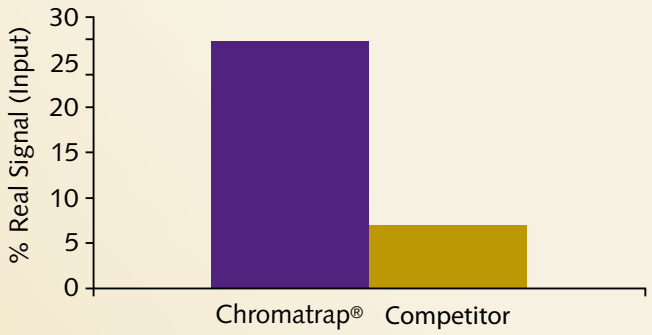
Gel electrophoresis of human FFPE tumour chromatin sheared using the Chromatrap® FFPE ChIP kit. Uniform chromatin fragments lengths between 100-500 bp ideal for ChIP have been achieved using the Chromatrap® FFPE ChIP kit.

The Chromatrap® FFPE ChIP kit has overcome the issues often associated with FFPE ChIP, by offering a streamlined protocol which provides the user with excellent quality isolated chromatin.

- **Wide dynamic range provides greater flexibility and more IPs per sample**
- **Works across a range of FFPE samples, human and animal**
- **Available in spin column or 96-well high throughput format**
- **Compatible with high and low abundant marks**
- **Provides sufficient DNA to perform library preps for ChIP-seq assays**

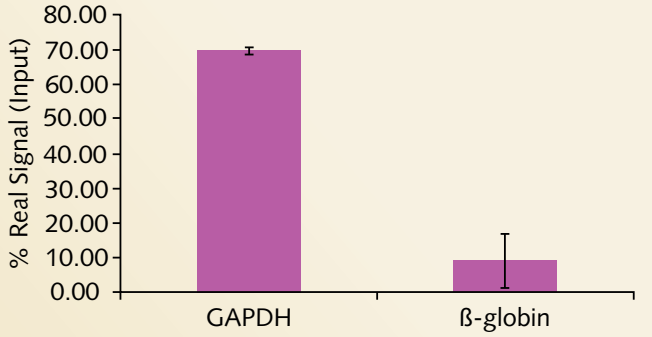
It is ideal for tissue samples, biopsies and difficult-to-lyse samples. The sensitivity of the unique solid-state platform ensures that small samples can be used with efficient enrichment of both high and low abundant targets. Chromatrap® FFPE ChIP kits provide a quicker, easier and more efficient way of performing ChIP assays from a range of FFPE samples. Using the unique, patented Chromatrap® technology maximises the capture efficiency of the target chromatin/antibody complex. By avoiding the use of magnetic or agarose beads, non-specific background is eliminated together with reduced manual handling errors and subsequent sample loss.

H3 enrichment of the *GAPDH* locus



Chromatin was extracted from rat FFPE uterine tissue and immunoprecipitated for the high abundant mark H3 using Chromatrap® Pro A FFPE ChIP kit and compared against a competitor kit. The graph shows level of enrichment of H3 onto the GADH locus to be 4x that of the leading competitor, with excellent signal to noise ratio.

H3K4me3 enrichment of the *GAPDH* and *ß-globin* loci



Human breast FFPE tumour tissue extracted and immunoprecipitated using Chromatrap® FFPE Pro A ChIP kit can be used for both high and low abundant marks on positive and negative gene targets.

Chromatrap® FFPE kits outperform when compared to traditional methods:

- **10x less starting material for qPCR**
- **Requires just 10 x 3 µm sections**
- **At least 4x better pull down than competitor kits**
- **No need to pool samples for sequencing**

The Chromatrap® FFPE ChIP kit is fully compatible with next generation sequencing. Sufficient DNA can be obtained from as little as a single IP for preparation of high quality NGS libraries with <10% duplication, even from the most difficult of samples.

QC validation	Chromatrap FFPE ChIP-seq
FAST Q	>30
H3K4me3 ChIP-seq	>14,000 genes identified
Peak alignment	Significantly enriched peaks (MACS); Compatible for TF and histone modification mapping

Products available from Chromatrap®

Product	Quantity	Catalogue no.
Chromatrap® ChIP-seq Pro A	24	500189
Chromatrap® ChIP-seq Pro G	24	500190
Chromatrap® HT ChIP-seq Pro A	1 x 96	500214
Chromatrap® HT ChIP-seq Pro G	1 x 96	500215
Chromatrap® Enzymatic ChIP-seq Pro A	24	500191
Chromatrap® Enzymatic ChIP-seq Pro G	24	500192
Chromatrap® HT Enzymatic ChIP-seq Pro A	1 x 96	500216
Chromatrap® HT Enzymatic ChIP-seq Pro G	1 x 96	500217
Chromatrap® ChIP qPCR Pro A	24	500071
Chromatrap® ChIP qPCR Pro G	24	500117
Chromatrap® Premium ChIP qPCR Pro A	24	500115
Chromatrap® Premium ChIP qPCR Pro G	24	500116
Chromatrap® HT ChIP qPCR Pro A	1 x 96	500161
Chromatrap® HT ChIP qPCR Pro G	1 x 96	500163
Chromatrap® HT Enzymatic ChIP qPCR Pro A	1 x 96	500162
Chromatrap® HT Enzymatic ChIP qPCR Pro G	1 x 96	500164
Chromatrap® Enzymatic ChIP qPCR Pro A	24	500166
Chromatrap® Enzymatic ChIP qPCR Pro G	24	500168
Chromatrap® Premium Enzymatic ChIP qPCR Pro A	24	500167
Chromatrap® Premium Enzymatic ChIP qPCR Pro G	24	500169
Chromatrap® FFPE ChIP-seq Pro A	24	500235
Chromatrap® FFPE ChIP-seq Pro G	24	500236
Chromatrap® Native ChIP-seq Pro A	24	500237
Chromatrap® Native ChIP-seq Pro G	24	500238
Chromatrap® Sonication Shearing		500239
Chromatrap® Enzymatic Shearing		500165
Chromatrap® DNA purification HT	2 x 96	500220
Chromatrap® DNA clean and concentrate HT	2 x 96	500240

Chromatrap

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