Chromatrap®

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

ADVANCEMENTS IN EPIGENETICS

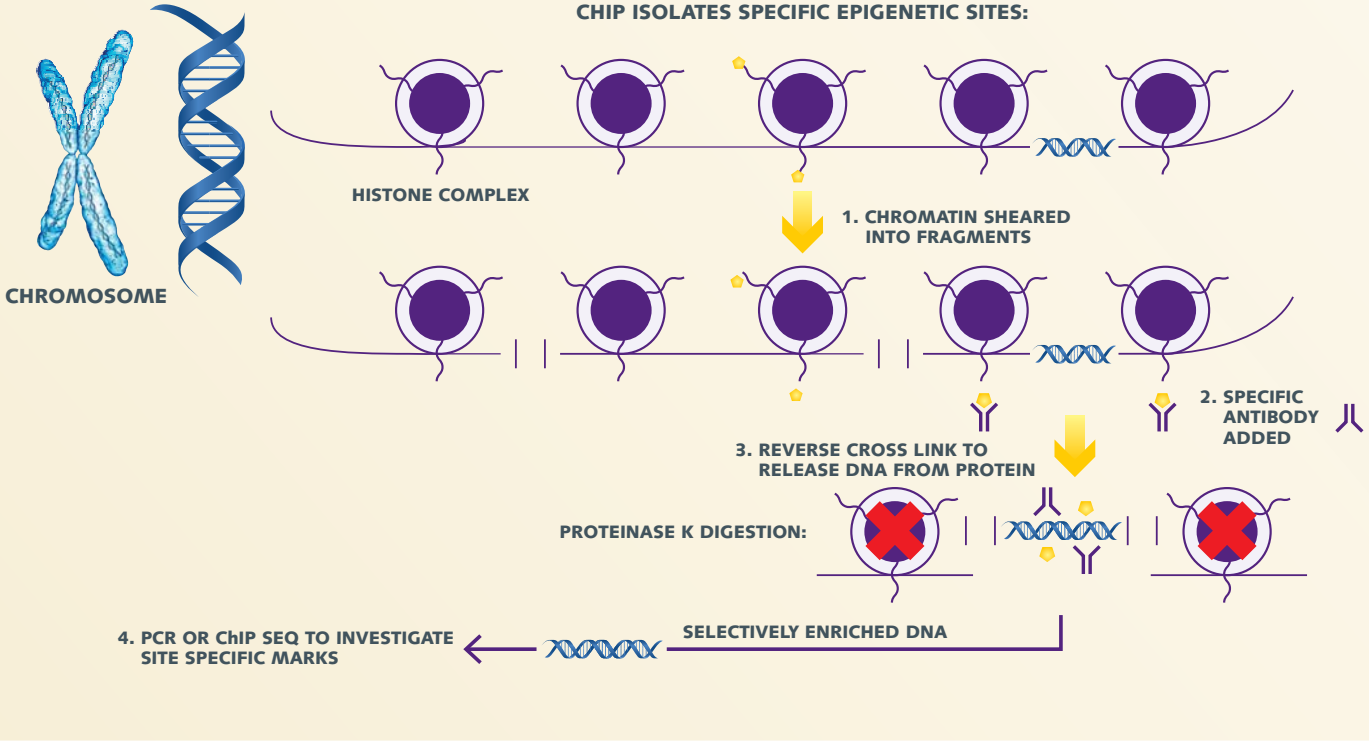
What is ChIP?

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.

Epigenetics is the study of the molecular mechanisms which control gene expression in a potentially heritable way that do not involve changes in the underlying DNA sequence. ChIP is used to study the association of specific proteins, or their modified isoforms, with defined genomic regions.

In a ChIP assay, cells are fixed to form cross-links which retain the specific DNA-protein interactions. This 'chromatin' is extracted and sheared by sonication or enzymatic digestion into small fragments. The fragments are selectively immunoprecipitated using antibodies against the protein of interest and the fractions treated to separate DNA and protein. PCR, RT-PCR, hybridisation on microarrays, or direct sequencing are used to identify DNA fragments of defined sequence.

ChIP is a rapidly growing research technique in epigenetics which is now starting to be applied to mechanism of action studies in drug discovery; stratified medicine and diagnostics, and also alongside DNA sequencing in ChIP-seq. Successful chromatin preparation and IP is critical to these processes. **Chromatrap® ChIP assay kits enhance both the preparation and performance of your ChIP assays**.



What is Chromatrap®?

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

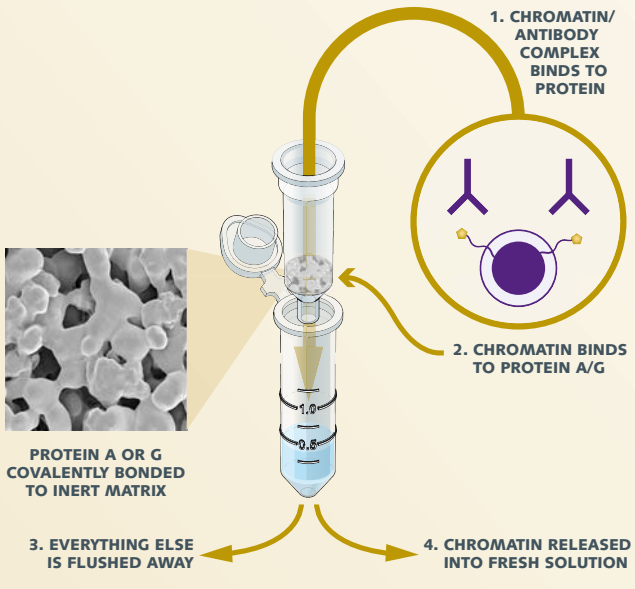
Compared to standard methods, Chromatrap® is:

- 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 a fast processing time of under five hours from chromatin loading to qPCR-ready DNA, Chromatrap® significantly reduces the assay time for ChIP, enabling more samples to be analysed during the working day and thereby 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. The latest ChIP-seq kits offer exceptional performance for Illumina® sequencing library preparation in half the time previously needed. Further exciting new developments in the Chromatrap® range are planned, so do keep up to date with our website at: **www.chromatrap.com**

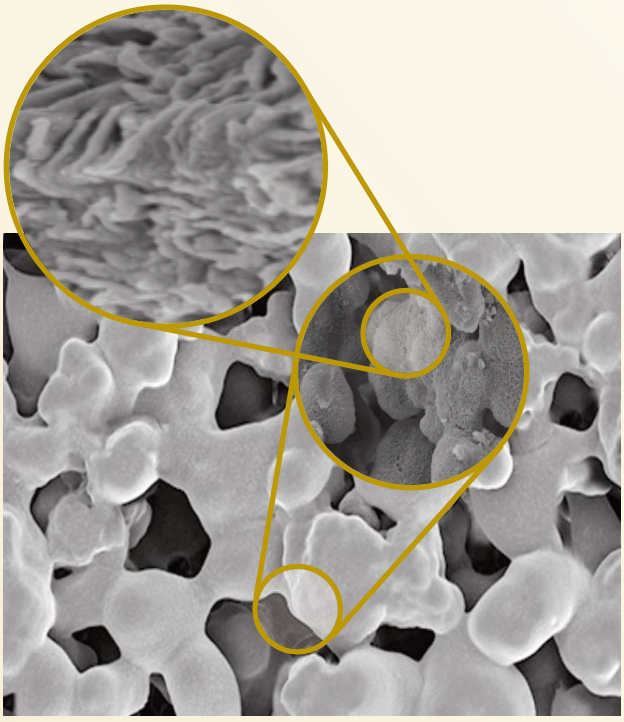
Chromatrap® offers an inert solid-phase scaffold for better immunoprecipitation



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

High surface area and flow-through characteristics are key to efficiency



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

Chromatrap® qPCR kits provide a more convenient, quicker and simpler method for performing ChIP assays in laboratories that do not require sequencing. These kits are cost effective and ideal where qPCR results alone are sufficient.

Chromatrap® qPCR kits outperform all other chromatin immunoprecipitation methods:

Robust signal to noise
- *Typically 2-3 times better than standard methods*

Low non-specific binding *of the inert Chromatrap® discs*
- *No pre-blocking step required*
- *No DNA clean-up required*

High surface area and excellent molecular mixing – *ideal for*
- *Low abundant targets*
- *Challenging cell types, including primary cells*

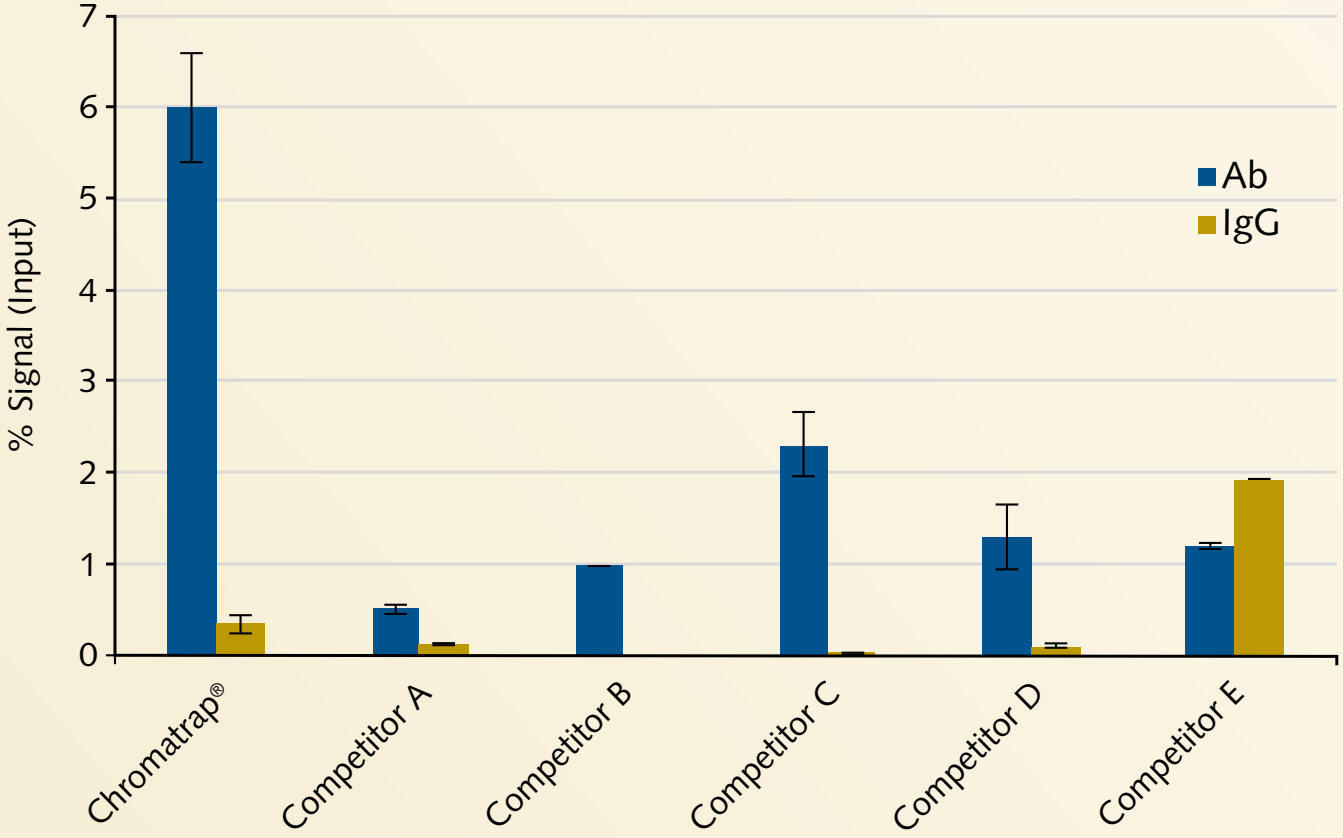
Excellent 'flow through' characteristics *of the column*
- *Easy, fast washing and elution steps during the assay*
- *Ensures highest levels of chromatin capture and release*

Wide dynamic range
- *From 50 ng to 7000 ng*
- *More ChIP assays from a single sample*

Reduced incubation steps

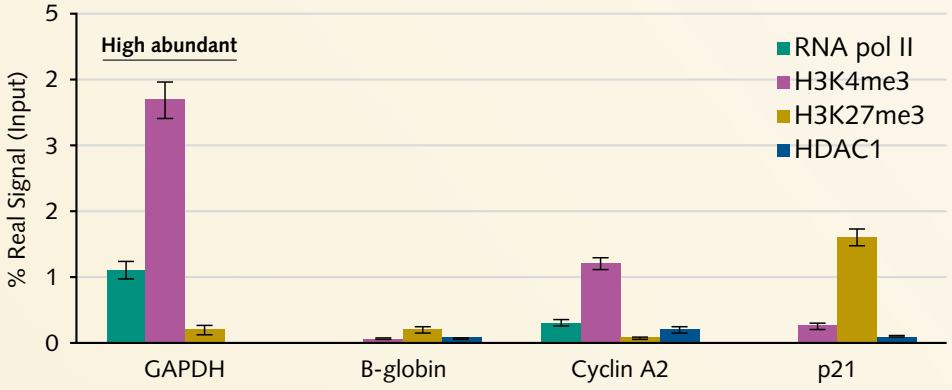
1: Ideal for low chromatin loadings: offers significant assay flexibility advantages

Chromatrap® has been compared under standardised conditions using 1 µg of chromatin sample in each case against five popular ChIP assay kits. The data shown below demonstrates excellent results for Chromatrap® under these standard conditions and starting with our recommended chromatin loading of 1 µg. Chromatrap® RECOMMENDS smaller input sample sizes of 50 ng-7000 ng, meaning that more assays are possible from one chromatin sample.



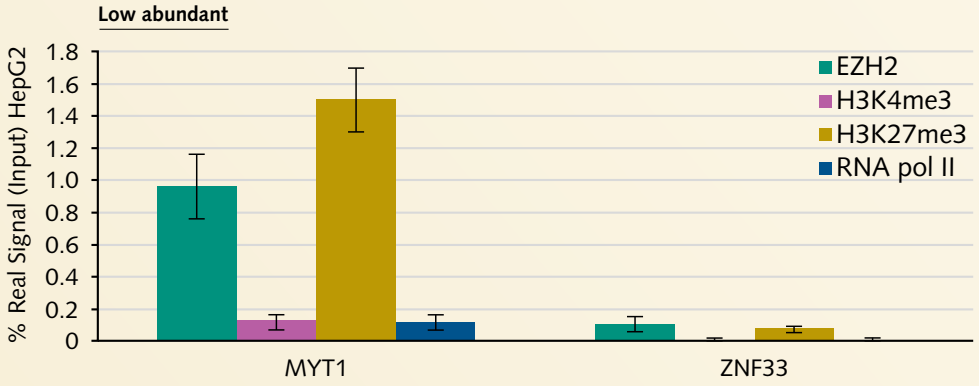
2: Signal strength: clear differentiation across the epigenetic landscape

The sensitivity and selectivity of Chromatrap® is clearly shown with data highlighting positive and negative gene targets alongside the epigenetic landscape for both high abundant and low abundant transcription factors.



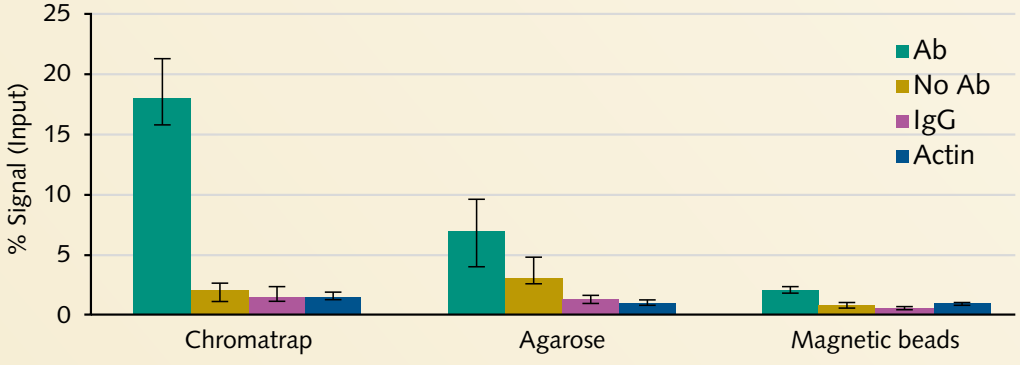
3: Signal strength: allows clear results from difficult or low abundant targets

Thanks to the high signal to noise and excellent selective chromatin capture and release, Chromatrap® is able to successfully differentiate between positive and negative gene targets even when these transcription factors are expressed with low abundance. The graph shows excellent correlation between H3K4me3 and H3K27me3 over positive and negative gene targets using a standard Chromatrap® spin column kit. This demonstrates the excellent sensitivity of standard Chromatrap® kits for transcription factors with low presence which may be difficult to assay with competitors' kits.



4: Robust signal to noise compared to traditional bead-based methods

The following data demonstrates the superiority of the Chromatrap® ChIP assay. In trials against magnetic and agarose beads, Chromatrap® is shown to have a significantly improved signal to noise, good reproducibility and excellent DNA enrichment.



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.

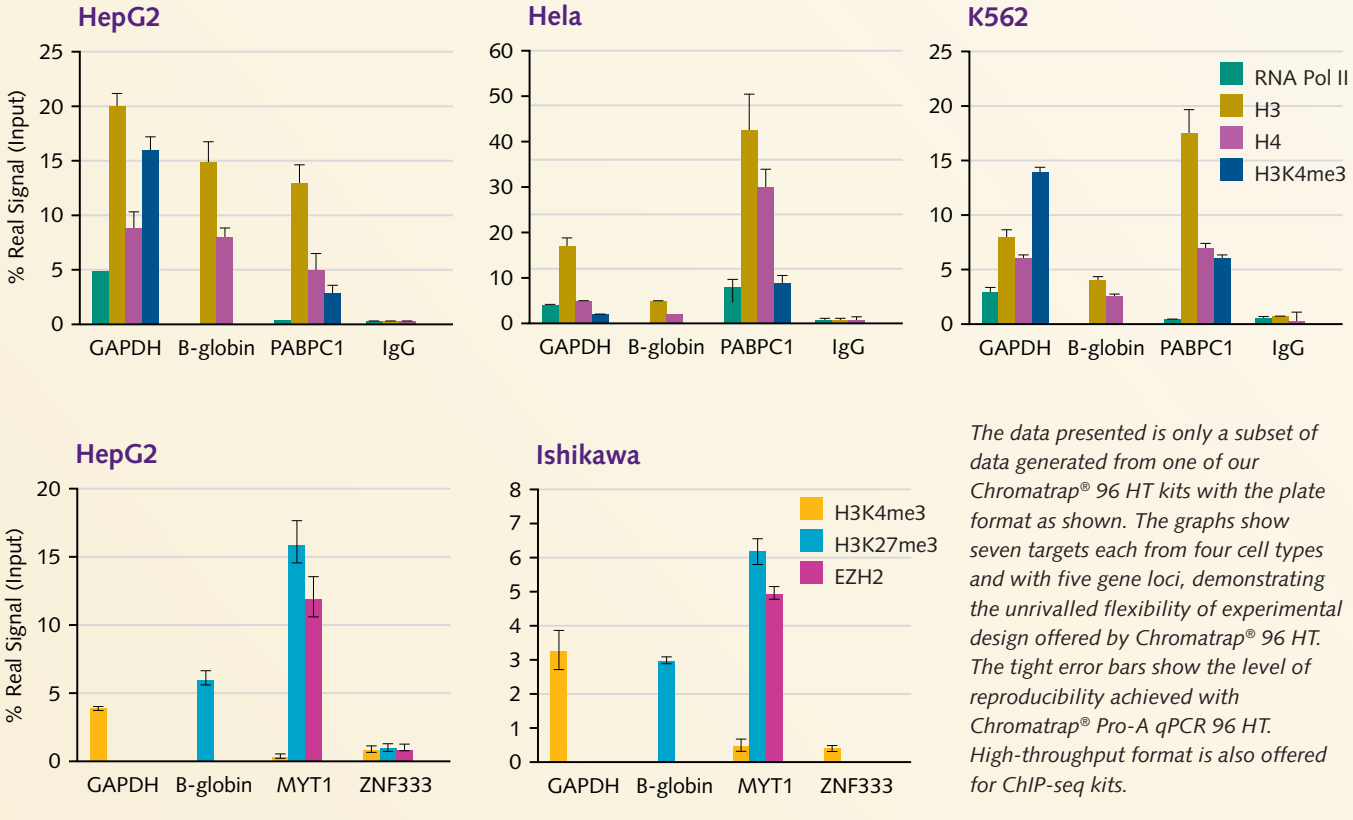
Example plate layout for 96 reactions

	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® ChIP-seq

Genome-wide mapping of protein-DNA interactions is essential for a complete understanding of gene regulation. The most widely used tool for examining these interactions is ChIP followed by massively parallel sequencing (ChIP-seq). With the ability to sequence tens to hundreds of millions of DNA fragments in a single run, ChIP-seq offers significantly more data compared to with previous approaches set to become the leading technology for genome-scale of protein-DNA interactions.

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.

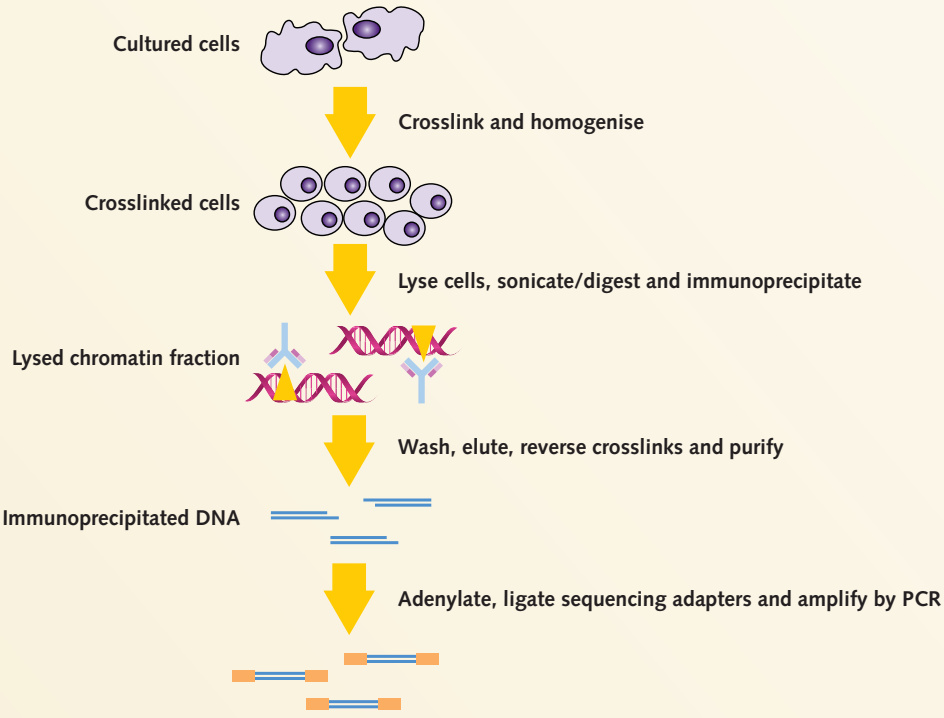
The Chromatrap® ChIP-seq kit has been optimised for use with Illumina® library preparation kits and sequencing platforms to deliver the greatest quantity of DNA for sequencing. ChIP sample processing time is greatly reduced relative to traditional bead-based assays, requiring just 5 hours and up to 500,000 cells per IP.

Thanks to Chromatrap's® unique solid-phase matrix and the option of a compact 96-well plate format, sample throughput and reproducibility is increased due to smaller sample volumes, centrifugal wash-steps and a matrix that does not require blocking.

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 10-50 g of chromatin
- Wide dynamic range allows lower chromatin loadings and minimises antibody requirements.
- Fully compatible with Illumina® MiSeq™ and HiSeq™
- Reduced IP incubation times

The data analysis tool employed in this study is currently under development for those customers who choose Chromatrap® for their ChIP-seq assay. This eliminates many complicated commands, which are instead replaced with an easy to use interface that allows intuitive analysis of your ChIP-seq dataset.

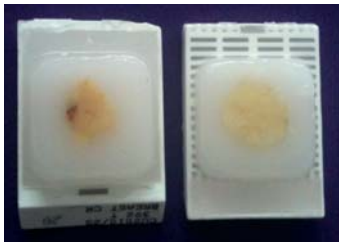


Overview of the ChIP-seq process with a graphical visualisation of read alignment

Chromatrap® FFPE

In most cancer patients, global epigenetic alterations have been observed which have been linked to clinical outcome of the disease. To define the role of such epigenetic alterations in human disease and to identify potential biomarkers, it is necessary to analyse samples from these patients, many of which will be presented as formaldehyde-fixed, paraffin embedded (FFPE) samples. Thus epigenetic identification and targeting in clinical FFPE tissues is a crucial step for potential epigenetic therapeutics and personalized medicine therapies.

FFPE is a standard method to archive and preserve medical tissue biopsy samples. These archives, kept in many hospitals world-wide, are a huge, rich and untapped sample resource for genetic research. Due to the cross-linking of proteins by formaldehyde, FFPE tissues present a particular challenge for ChIP analysis.



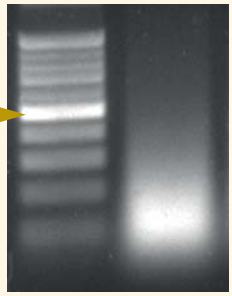
FFPE tissue block

In addition, the small size and delicate nature of the tissue, and the difficulty in extracting samples from paraffin, used to provide tissue support after preservation, can damage complex cellular complexes such as chromatin. ChIP assays from these samples have therefore proved time consuming and difficult.

Chromatrap® have overcome these issues and provide a Chromatrap® FFPE ChIP kit which:

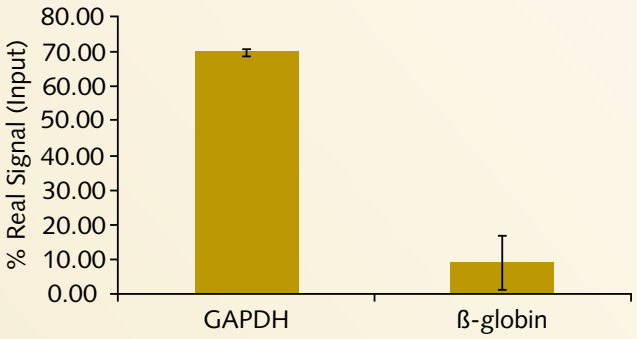
- **Provides greater flexibility and more IPs per sample**
- **Works across a range of FFPE samples, human and animal**
- **Available in 96-well plate or spin column format**
- **Works equally well with high and low abundant marks**
- **Provides sufficient DNA to perform library preps for ChIP-seq assays**

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.

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

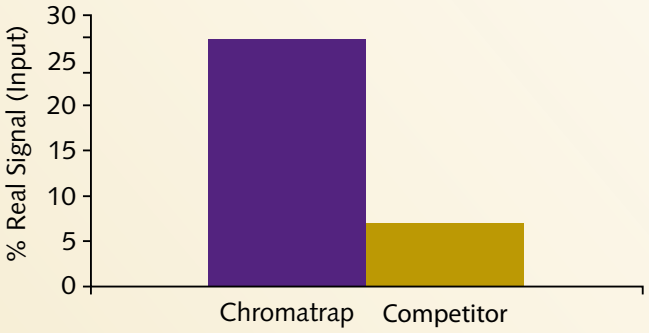


Human breast FFPE tumour tissue extracted and immunoprecipitated using Chromatrap® FFPE Pro A ChIP kit can be used for both high and low abundant marks on positive and negative gene targets.

Chromatrap® FFPE kits outperform when compared to traditional methods:

- **10x less starting material**
- **Requires just 10 3 µm sections**
- **At least 4x better pull down than competitor kits**

H3 enrichment of the *GAPDH* locus



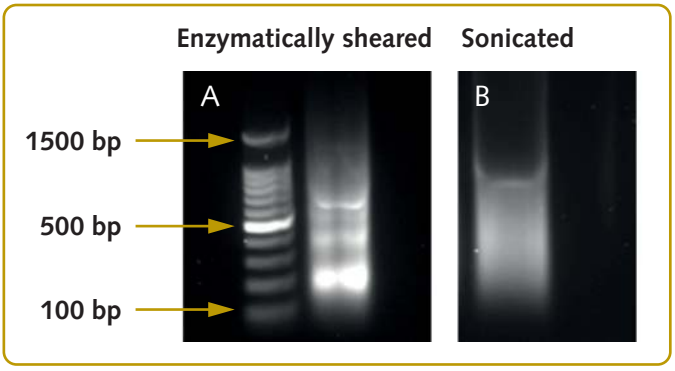
Chromatin was extracted from rat FFPE uterine tissue and immunoprecipitated for the high abundant mark H3 using Chromatrap® Pro A FFPE ChIP kit and compared against a competitor kit. The graph shows level of enrichment of H3 onto the GADH locus to be 4x that of the leading competitor, with excellent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

For more information on Chromatrap® FFPE ChIP kit please visit our website www.chromatrap.com or contact technical support support@chromatrap.com

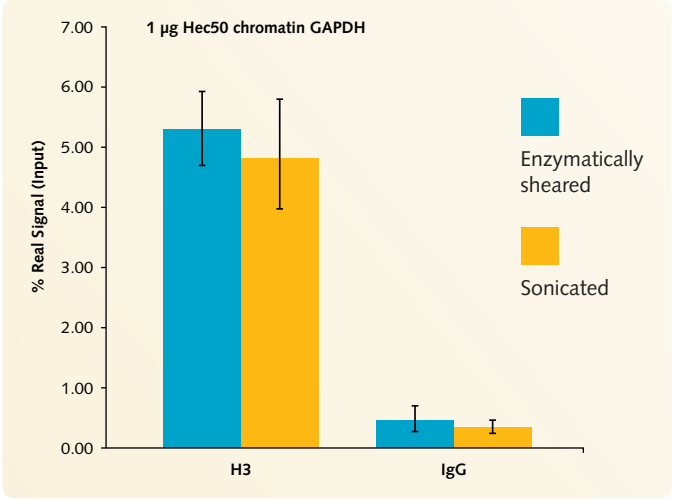
Enzymatic shearing

Chromatrap® enzymatic shearing kit

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.



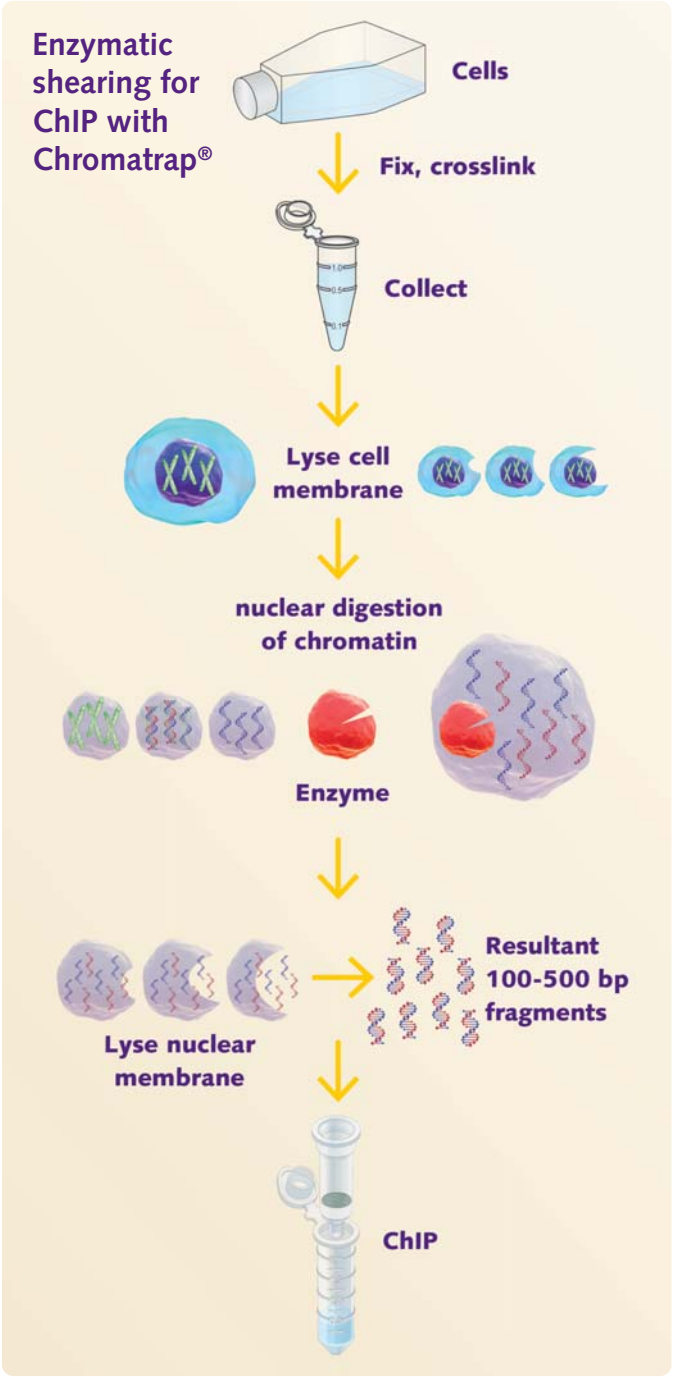
Gel electrophoresis of Hec50 chromatin using the Chromatrap® Enzymatic Shearing Kit and Spin Column Sonication Kit. *The banding pattern, typical of enzymatic digestion, with fragments of 200 bp, 400 bp and 600 bp – ideal for ChIP using Chromatrap® (A). Uniform chromatin fragment lengths between 100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

The Chromatrap® Enzymatic Shearing Kit provides an excellent method for the preparation of high quality, ideally fragmented chromatin for ChIP analysis. The kit demonstrates excellent enrichment, independent of starting cell number. With its quick and simple protocol the Chromatrap® Enzymatic Shearing Kit is the perfect cost-effective alternative to sonication.

The 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.



A more efficient, sensitive and robust method of chromatin immunoprecipitation

Offering novel technology that saves time, gives better results and is easier to use, Chromatrap® in both spin column and microplate formats is the ChIP method of the future. With options for sonication or enzymatic shearing, on Pro A or Pro G, Chromatrap® is the logical choice for epigenetics researchers everywhere.

Chromatrap® formats

ChIP Kits for qPCR – 24 spin columns
Standard kits contain all the buffers and filter materials, but do not contain any positive or negative control antibodies. Premium Kits contain both a positive and a negative control antibody along with a positive primer set, validated with Chromatrap® ChIP assays to give you added peace of mind. These kits are highly recommended for users who are contemplating ChIP for the first time, as the control standards identify a potential source of error in the experiment. These kits are available in enzymatic format for those who do not have access to a sonicator. All are available as Pro A or Pro G.

ChIP Kits for Sequencing (ChIP-seq) – 24 spin columns
All ChIP-seq kits come with control antibodies and are available as Pro A or Pro G, Enzymatic or Sonication. ChIP-seq kits are compatible with Illumina® library preps.

High throughput ChIP 96-HT Kits – 96-well plate
A 96-well filter plate containing the Chromatrap® chemistry is used to process samples in high-throughput. Available as Standard or Premium, for qPCR or Sequencing, Pro A or Pro G, Enzymatic or Sonication.

Chromatrap® Enzymatic Shearing kit
The Chromatrap® Enzymatic shearing kit supplies all the necessary reagents and buffers for up to 10 chromatin preparations. This kit allows you to determine optimal shearing conditions for your chromatin preparations and can be used with the regular Chromatrap® ChIP-qPCR and ChIP-seq kits.

Ordering information

Part No.	Description	Pro A or Pro G	Qty	Prem.	Enzy.	For Seq?
500189	ChIP-seq 24 spin columns, Pro A, with Premium control antibodies	A	24	Y		Y
500190	**ChIP-seq 24 spin columns, Pro G, with Premium control antibodies**	**G**	**24**	**Y**		**Y**
500214	ChIP-seq 96-well plate kit Pro A, with Premium control antibodies	A	96	Y		Y
500215	**ChIP-seq 96-well plate kit Pro G, with Premium control antibodies**	**G**	**96**	**Y**		**Y**
500191	ChIP-seq Enzymatic 24 spin columns, Pro A, with Premium control antibodies	A	24	Y	Y	Y
500192	**ChIP-seq Enzymatic 24 spin columns, Pro G, with Premium control antibodies**	**G**	**24**	**Y**	**Y**	**Y**
500216	ChIP-seq Enzymatic 96-well plate kit Pro A, with Premium control antibodies	A	96	Y	Y	Y
500217	**ChIP-seq Enzymatic 96-well plate kit Pro G, with Premium control antibodies**	**G**	**96**	**Y**	**Y**	**Y**
500071	qPCR Standard Chromatrap Pro A spin column kit for 24 samples	A	24			N
500117	**qPCR Standard Chromatrap Pro G spin column kit for 24 samples**	**G**	**24**			**N**
500115	qPCR Premium Chromatrap Pro A spin column kit, 24 columns	A	24	Y		N
500116	**qPCR Premium Chromatrap Pro G spin column kit, 24 coloumns**	**G**	**24**	**Y**		**N**
500161	qPCR Standard Chromatrap Pro A high throughput ChIP 96-well Microplate	A	96			N
500163	**qPCR Standard Chromatrap Pro G high throughput ChIP 96-well Microplate**	**G**	**96**			**N**
500162	qPCR Enzymatic Standard Chromatrap Pro A high throughput ChIP 96-well Microplate	A	96		Y	N
500164	**qPCR Enzymatic Standard Chromatrap Pro G high throughput ChIP 96-well Microplate**	**G**	**96**		**Y**	**N**
500166	qPCR Enzymatic Standard Chromatrap Pro A spin column kit for 24 samples	A	24		Y	N
500168	**qPCR Enzymatic Standard Chromatrap Pro G spin column kit for 24 samples**	**G**	**24**		**Y**	**N**
500167	qPCR Enzymatic Premium Chromatrap Pro A spin column kit, 24 columns	A	24	Y	Y	N
500169	**qPCR Enzymatic Premium Chromatrap Pro G spin column kit, 24 coloumns**	**G**	**24**	**Y**	**Y**	**N**
500165	Enzymatic Buffers kit				Y	N

Chromatrap®

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