ab156907 – ChIP Kit Magnetic – One-Step

Instructions for Use

For selective enrichment of a chromatin fraction containing specific DNA sequences in a high throughput format using chromatin isolated from various species, particularly mammals

This product is for research use only and is not intended for diagnostic use.

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Table of Contents

1. BACKGROUND

Protein-DNA interaction plays a critical role for cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, and epigenetic silencing. Identifying the genetic targets of DNA binding proteins and knowing the mechanisms of protein-DNA interaction is important for understanding cellular process.

Chromatin immunoprecipitation (ChIP) offers an advantageous tool for studying protein-DNA interactions. It allows for the detection that a specific protein binds to the specific sequences of a gene in living cells by PCR (ChIP-PCR), microarrays (ChIP-chip), or sequencing (ChIP-seq). For example, measurement of the amount of methylated histone H3 at lysine 9 (meH3-K9) associated with a specific gene promoter region under various conditions can be achieved through a ChIP-PCR assay, while the recruitment of meH3K9 to the promoters on a genome-wide scale can be detected by ChIP-chip. In particular, ChIP with antibodies directly against various transcriptional factors is widely demanded.

However, currently used ChIP methods have several drawbacks of which the most critical weakness is lengthy procedures, often taking up to 3 days to finish the procedures. Additionally, the labor-intensive procedure involves an excessive amount of steps, inconsistency, and sub-optimized chromatin shearing. These flaws result in inconvenience, low throughput processing, and less enrichment efficiency.

Because the major features of next generation sequencing and microarrays are their rapidness and high throughput capabilities, these technologies are becoming major players in massive protein-DNA analysis. To be compatible with these new technologies, rapid and massive generation of target protein-bound DNA is critically required.

To meet this requirement, Abcam's ChIP Kit Magnetic - One-Step has the following advantages:

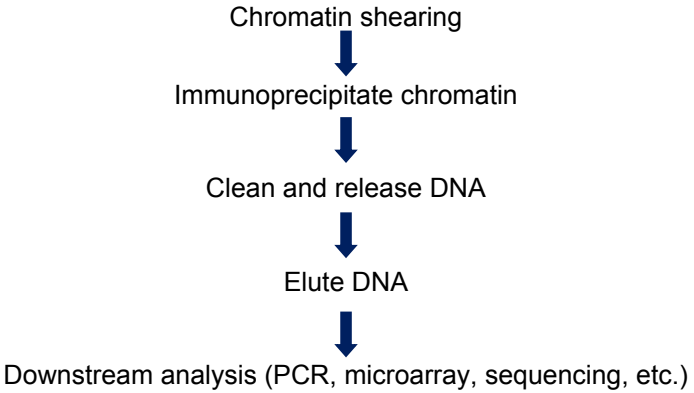
- 96-well plate format makes the assay flexible. Either (a) manual with one single reaction each time; or (b) high throughput with 96 reactions each time
- Highly efficient enrichment. Enrichment ratio of positive to negative control > 120, and an extremely low number of cells required (as low as 10,000 cells per ChIP reaction)
- High reproducibility. Pre-optimized ChIP conditions and reaction processing in sealed vials make the ChIP procedure consistent
- Wide downstream analysis compatibility. Compatible with various downstream analysis workflows including ChIP-PCR, ChIP-on-chip, and ChIP-seq

Abcam's ChIP Kit Magnetic - One-Step is suitable for selective enrichment of a chromatin fraction containing specific DNA sequences in a high throughput format using chromatin isolated from various species, particularly mammals. Chromatin can be isolated by using your own successful method or, for your convenience and the best results, with Abcam's Chromatin Extraction Kit (ab117152) optimized for use with this product. The target protein bound DNA prepared with Abcam's ChIP Kit Magnetic - One-Step can be used for various downstream applications including PCR (ChIP-PCR), microarrays (ChIP-chip), and sequencing (ChIP-seq).

ab156907 contains all necessary reagents required for carrying out a successful chromatin immunoprecipitation directly from chromatin extracts isolated from mammalian cells or tissues. This kit includes a positive control antibody (RNA polymerase II), a negative control non-immune IgG, and GAPDH primers that can be used as a positive control to demonstrate the efficacy of the kit reagents and protocol. RNA polymerase II is considered to be enriched in the GAPDH gene promoter that is expected to be undergoing transcription in most growing mammalian cells and can be immunoprecipitated by RNA polymerase II but not by non-immune IgG. Immunoprecipitated DNA is then cleaned, released, and eluted. Eluted DNA

can be used for various downstream applications such as ChIP-PCR, ChIP-on-chip, and ChIP-seq.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table and away from light upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if the 10X Wash Buffer contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

5. MATERIALS SUPPLIED

Item	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	10 mL	20 mL	4°C
ChIP Buffer	6 mL	12 mL	RT
DNA Release Buffer	14 mL	28 mL	RT
Non-Immune IgG (1 mg/mL)	10 µL	20 µL	4°C
Anti-RNA Polymerase II (1 mg/mL)	8 µL	16 µL	4°C
Proteinase K (10 mg/mL)	55 µL	110 µL	4°C
GAPDH Primer - Forward (20 µM)	8 µL	16 µL	4°C
GAPDH Primer - Reverse (20 µM)	8 µL	16 µL	4°C
8-Well Strip Caps	12	24	RT
96-Well PCR Plate	2	2	RT
Magnetic Beads	220 µL	450 µL	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Variable temperature waterbath or incubator oven
- Thermocycler with 48 or 96-well block
- Orbital shaker
- Magnetic stand (96-well format)
- Adjustable pipette or multiple-channel pipette
- Aerosol resistant pipette tips
- 0.2 mL or 0.5 mL PCR vials
- Antibodies of interest
- Microplate centrifuge
- Rolling shaker

Note: *Antibodies should be ChIP or IP grade as to recognize fixed and native proteins that are bound to DNA or other proteins. If you are using antibodies which have not been validated for ChIP, then appropriate control antibodies such as RNA Polymerase II should be used to demonstrate that the antibody and chromatin are suitable for ChIP.*

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

1X Wash Buffer

Add the volume specified in the table below of 10X Wash Buffer to distilled water (pH 7.2-7.5).

	Volume to Dilute (mL)	Volume distilled water (mL)	Total Volume (mL)
48 Tests	**8**	**72**	**80**
96 Tests	**16**	**144**	**160**

The 1X Wash Buffer can be stored at 4°C for up to six months.

10. SAMPLE PREPARATION

Input Chromatin Amount: Chromatin amount can range from 0.1-15 µg per reaction. AN optimal amount is 5-10 µg per reaction.

Chromatin Isolation: You can use your method of choice for chromatin isolation. Abcam offers the Chromatin Extraction Kit (ab117152) for your convenience. Isolated chromatin can be stored at -20°C or -80°C (long term) until use.

Chromatin Shearing: If a probe-based sonicator will be used, the sonication settings need to be optimized by you. An example protocol to obtain DNA of 200-1000 bp size:

For example, DNA of 200-1000 bp size can be obtained by sonicating 3-4 pulses of 15-20 seconds each at 40% output power using a microtip probe, followed by a 30-40 seconds rest period on ice between each pulse. If desired, remove 10 µL of sheared chromatin for DNA purification and agarose gel analysis along with a DNA marker on a 1.5% agarose gel, stained with Ethidium bromide, and visualize it under ultraviolet light.

11. ASSAY PROCEDURE

11.1 Preparation of One-Step ChIP Reaction

11.1.1 Predetermine the number of PCR wells required for your experiment. Cap the unused wells of the 96-Well PCR Plate with the supplied 8-Well Strips Caps before continuing further to avoid any possible contamination. Ensure the tubes are sealed. Plate can also be saved for later use.

11.1.2 Setup the one-step ChIP reactions by adding the reagents to each well according to the following:

Reagents	Sample (µL)	Positive control (µL)	Negative control (µL)
ChIP Buffer	55-75	55-75	55-75
Chromatin	20-40	20-40	20-40
Your Antibody of Interest	0.5 - 2	0	0
RNA Polymerase II	0	0.8	0
Non-Immune IgG	0	0	0.8
Magnetic Beads	4	4	4
Total Volume	**100**	**100**	**100**

***Note**: The final amount of each component should be (a) chromatin: 5-10 µg/well; (b) antibodies of interest: 0.8 µg/well; (c) RNA Polymerase II: 0.8 µg/well; and (d) non-immune IgG: 0.8 µg/well.*

Freshly prepared chromatin can be used directly for the reaction. Frozen chromatin samples should be thawed quickly at RT and then placed on ice before use. Store remaining chromatin samples at -20°C, or at -80°C if they will not be used within 8 hours.

The amounts of the positive control and negative control are sufficient for matched use with samples if two antibodies are used for each sample or one antibody is used for two of the same samples. If using one antibody of interest for each sample with matched use of the positive and negative control, extra RNA polymerase II and non-immune IgG may be required.

Input DNA control is only used for estimating the enrichment efficiency of ChIP and is generally not necessary as the positive and negative control can be used for estimating the same objective more accurately.
If you would like to include the input DNA control, the following steps can be carried out:
(1) add 10 µL of each chromatin sample to a 0.2 mL PCR tube followed by adding 88 µL of DNA Release Buffer and 2.5 µL of Proteinase K;
(2) incubate the input DNA control at 65°C for 15 minutes, then incubate at 95°C for 10 minutes;
(3) spin the solution down to the bottom. Input DNA is ready for PCR or storage at -20°C.

11.1.3 Cap the reaction wells with the 8-Well Strip Caps and place the 96-Well PCR Plate on a rolling shaker for 120 minutes. Tape the plate to the rolling shaker securely. Alternatively, the sample can be transferred to microfuge tubes if you prefer not to do this step using a plate.

11.1.4 Remove the plate from the rolling shaker. If liquid is found in the caps, briefly spin the plate down using a microplate centrifuge.

11.2 **Washing of the Reaction Wells**

11.2.1 Carefully remove the caps from the reaction wells.

11.2.2 Place the plate on a magnetic stand (96 well format) for 1-2 minutes in order to pellet the beads to the sides of the wells.

11.2.3 Carefully remove and discard the solution from each reaction well.

11.2.4 Wash each reaction well four times with 180 µL of 1X Wash Buffer each time. Each wash can be performed as follows:

11.2.4.1 Remove the plate from the magnetic stand (after solution has been removed).

11.2.4.2 Add 1X Wash Buffer to the reaction wells. Resuspend the beads by gently pipetting up and down several times. Ensure the pellet is completely resuspended and beads are not clinging to the pipette tips after pipetting.

11.2.4.3 Place the plate back to the magnetic stand for 1-2 minutes to pellet the beads and then remove and discard the solution from each reaction well.

11.2.5 Wash each reaction well one time with 180 µL of DNA Release Buffer with the same wash procedure as above (using DNA Release Buffer instead of 1X Wash Buffer).

11.2.6 Remove and discard the DNA Release Buffer solution from each well.

11.2.7 Remove the plate from the magnetic stand.

11.3 **Reversal of Cross-Links, Release and Elution of DNA**

11.3.1 Prepare the DNA Release Buffer-PK solution by adding 1 µL of Proteinase K to each 39 µL of DNA Release Buffer, and mix.

11.3.2 Add 40 µL of the DNA Release Buffer-PK solution to each reaction well, then cover with new strip caps.

11.3.3 Incubate the wells at 65°C for 15 minutes and then 95ºC for 5 minutes in a thermal cycler.

11.3.4 Remove the plate to room temperature. If liquid is collected on the inside of the caps, briefly spin the liquid down to the bottom.

11.3.5 Place the plate on the magnetic stand (96 well format) for 1-2 minutes to pellet the beads to the sides of the tubes/wells.

11.3.6 Carefully transfer the supernatant from each reaction well to new 0.2 or 0.5 mL PCR tubes. Cap the PCR tubes.

Purified DNA is now ready for use or storage at -20°C.

For real time PCR analysis, we recommend the use of 1-2 µL of eluted DNA in a 20 µL PCR reaction. If input DNA will be used, it should be diluted 10

fold before adding to PCR reaction. Control primers (110 bp, for human cells) included in the kit can be used as a positive control.

For end point PCR, the number of PCR cycles may need to be optimized for better PCR results.
In general, the amplification difference between “normal IgG control” and “positive control” may vary from 3 to 8 cycles, depending on experimental conditions.

For ChIP-chip or ChIP-seq, additional DNA clean up and concentration steps may be needed. For your convenience, Abcam offers a DNA Concentrator Kit (ab156895) for DNA clean up and concentration.

12. ANALYSIS

Typical Results

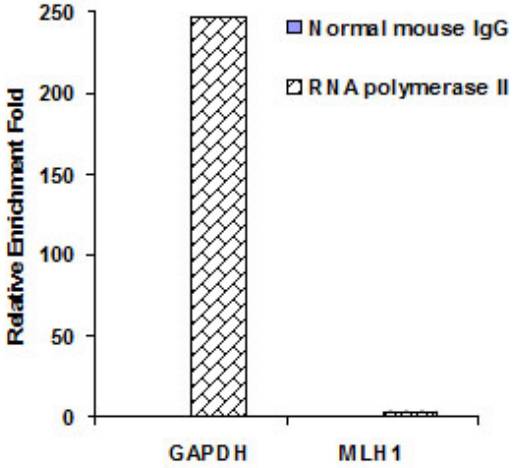


Fig 1. The data above shows the analysis of enrichment of RNA polymerase II in GAPDH and MLH1 promoters by Abcam's ChIP Kit Magnetic - One-Step (an156907), with chromatin extract prepared from formaldehyde fixed colon cancer cells. Captured DNA was used for analyzing levels of RNA polymerase II enriched in the GAPDH and MLH1 promoters.

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13. TROUBLESHOOTING

Problem	Cause	Solution
Little or No PCR Products Generated from both Sample and Positive Control Wells	Poor chromatin quality due to insufficient amount of cells, or insufficient or over cross-linking	To obtain an optimal amount of chromatin per ChIP reaction should be 5-10 µg (about 0.5-10^6 cells). The minimum amount of chomatin is 0.05 µg (5,000 cells). Appropriate chromatin cross-linking is also required. Insufficient or over-crosslinking will cause DNA loss or increased background. During cross-linking step of chromatin preparation, ensure that the cross-linking time is within 10-15 minutes, the concentration of formaldehyde is 1% as the final concentration, and/or quench solution is 0.125 M glycine
	Poor enrichment with antibody; some antibodies used in ChIP might not efficiently recognize fixed protein	Increase the antibody amount and use ChIP-grade antibodies validated for use in ChIP

	Inappropriate DNA fragmenting condition	If chromatin is from specific cell/tissue types such as plant, or is differently fixed, the processing program must be modified (see sonicator manual) to optimize the processing results If using a probe-based sonicator, shearing conditions should also be optimized to allow DNA fragment size to be between 200-1000 bp
	Incorrect temperature and/or insufficient time during DNA release	Ensure the incubation times and temperatures described in the protocol are followed correctly
	Improper PCR conditions, including improper PCR programming, PCR reaction solutions, and/or primers	Ensure the PCR is properly programmed. If using a homebrew PCR reaction solution, check if each component is correctly mixed. If using a PCR commercial kit, check if it is suitable for your PCR. Confirm species specificity of primers. Primers should be designed to cover a short sequence region (70-150 bp) for more efficient and precise amplification of target DNA region 9 binding sites of the protein of interest

	Improper sample storage	Chromatin sample should be stored at -80°C for no longer than 6 months, preferably less than 3 months. Avoid repeated freeze/thaw cycles DNA samples should be stored at -20°C for no longer than 6 months, preferably less than 3 months
No Difference in Signal Intensity Between Negative and Positive Control Wells	Insufficient washing	Check if washing recommendations at each step is performed according to the protocol. If the signal intensity in the negative control is still high, washing stringency can be increased in the following ways: 1. Increase wash time at each wash step: after adding 1X Wash Buffer, leave it in the tubes/wells for 2-3 minutes and then remove it 2. Add an additional one to two washes: The provided volume of 1X Wash Buffer is sufficient for 4 extra washes for each sample

	Too many PCR cycles; Plateau phase of amplification caused by over-increased number of PCR cycles in endpoint PCR may mask the difference of signal intensity between negative control and positive control	Decrease the number of PCR cycles (e.g. 32-35 cycles) to keep amplification at the exponential phase will reduce high background in endpoint PCR and allow differences in amplification to be seen. Real time PCR is another choice in such cases
Little or No PCR Products Generated From Sample Wells Only	Poor enrichment with antibody; some antibodies used in ChIP might not efficiently recognize fixed protein	Increase the antibody amount and use ChIP-grade antibodies validated for use in ChIP
	PCR primers are not optimized	Confirm species specificity of primers. Primers should be designed to cover a short sequence region (70-150 bp) for more efficient and precise amplification of target DNA region 9 binding sites of the protein of interest

14. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)