

ab117138 – ChIP Kit – One Step

Instructions for Use

For carrying out a successful chromatin immunoprecipitation directly from chromatin extracts isolated from mammalian cells or tissues

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

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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 (H3K9me) associated with a specific gene promoter region under various conditions can be achieved through a ChIP-PCR assay, while the recruitment of H3K9me 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.

Abcam's ChIP Kit - 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 - One Step - 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 antibody
- GAPDH primers (positive control)

The GAPDH primers can be used as control to demonstrate the efficacy of the kit reagents. RNA polymerase II is considered to be enriched in the GAPDH gene promoter, expected to be undergoing transcription in most growing mammalian cells, and it can be immunoprecipitated by the RNA polymerase II antibody 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.

Abcam's ChIP Kit - 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 - One Step - can be used for various downstream applications including PCR (ChIP-PCR), microarrays (ChIP-chip), and sequencing (ChIP-seq).

The amount of chromatin for each reaction can be 0.1 µg (about 1 x 10⁴ cells) to 15 µg (about 1.5 x 10⁶ cells). For an optimal reaction, the

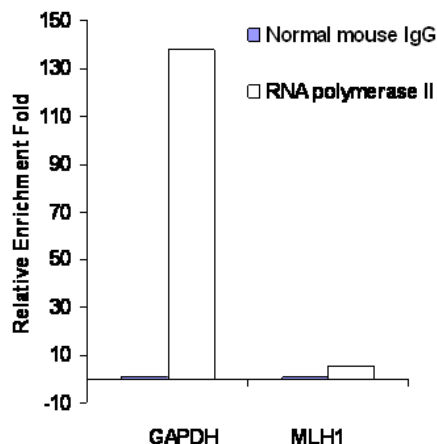
input chromatin amount should be 5 to 10 μg (about 0.5 to 1 x 10⁶ cells), as enrichment of target proteins to genome loci varies and some of the target proteins are of low abundance.

Starting materials can include various tissue or cell samples such as cells from flask or microplate cultured cells, fresh and frozen tissues.

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.

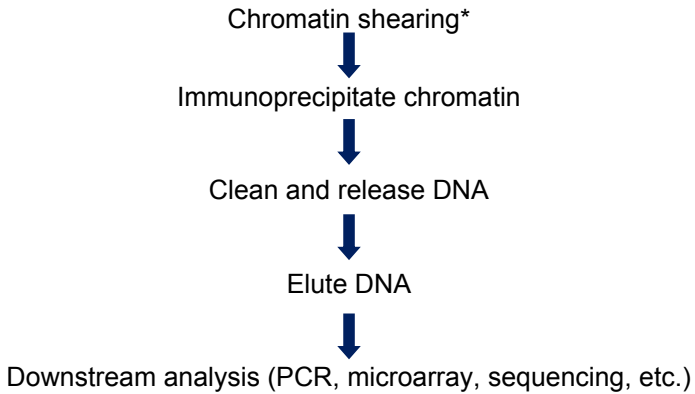
Both negative and positive DNA controls are provided in this kit.

To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.



The data above shows the analysis of enrichment of RNA polymerase II in GAPDH and MLH1 promoters by Abcam's ChIP Kit - One Step, 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.

2. ASSAY SUMMARY



*Chromatin shearing can be done

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 Assay Strips (With 1 Frame)	6	12	4°C
8-Well Strip Caps	6	12	RT
96-Well PCR Plate	1	1	RT
Adhesive Covering Film	1	2	RT
Sonication Frame*	1	1	RT

*To be used only with a high-throughput sonication instrument.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Variable temperature water bath or incubator oven
- Thermal cycler with 48 or 96-well block
- Adjustable pipette or multiple-channel pipette
- Aerosol resistant pipette tips
- 0.2 mL or 0.5 mL PCR vials
- Antibodies of interest
- Orbital shaker
- (Optional): HTP sonication instrument

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 10 mL of 10X Wash Buffer to 90 mL of distilled water (pH 7.2-7.5). 1X Wash Buffer can be stored at 4°C for up to six months.

10. SAMPLE PREPARATION

Input Chromatin Amount: Chromatin input 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 (short term), 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.

For example, DNA of 200-1000 bp size can be obtained by sonicating 3-4 pulses of 10-12 seconds each at level 2 using a microtip probe, followed by a 30-40 second 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 ChIP Reaction

11.1.1 Predetermine the number of strip wells required for your experiment. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

11.1.2 Setup the ChIP reactions by adding the reagents to each well according to the following chart:

Reagents	Sample (μL)	Positive control (μL)	Negative control (μL)
ChIP Buffer	50-60	50-60	50-60
Chromatin	40-50	40-50	40-50
Your Antibodies	0.5-2	0	0
RNA Polymerase	0	0.8	0
Non-Immune IgG	0	0	0.8

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 directly used 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 -80°C if they will be not used within 8 hours. The amounts of the positive control and negative control are sufficient for matched use with samples if two antibodies 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 μ L of DNA Release Buffer and 2.5 μ L of Proteinase K; (2) incubate the input DNA control at 65°C for 15 minutes followed by incubating at 95°C for 10 minutes; and (3) spin the solution down to the bottom. Input DNA is ready for PCR or storage at -20°C.

- 11.1.3 Seal the wells with Adhesive Covering Film and incubate the wells at room temperature for 90-120 minutes on an orbital shaker (100 rpm).
- 11.1.4 Peel away the Adhesive Covering Film carefully to avoid contamination between each well.

11.2 Washing of the Reaction Wells

- 11.2.1 Carefully remove the solution and discard from each well.
- 11.2.2 Wash each well with 200 μ L of 1X Wash Buffer each time for a total of 4 washes. This can be done by simply pipetting 1X Wash Buffer in and out of the well.
- 11.2.3 Wash each well with 200 μ L of the DNA Release Buffer one time by pipetting DNA Release Buffer in and out of the well.

11.3 Reversal of Cross-Links, Release and Purification of DNA

- 11.3.1 Prepare the DNA Release-PK solution by adding 1 μ L of Proteinase K to each 39 μ L of the DNA Release Buffer and mix.
- 11.3.2 Add 40 μ L of the DNA Release-PK solution to each well, then cover with Strip Caps.
- 11.3.3 Incubate the wells at 65°C for 15-20 minutes.
- 11.3.4 Quickly transfer the DNA solution from each well to 0.2 mL strip PCR tubes. Cap the PCR tubes.
- 11.3.5 Incubate the PCR tubes containing DNA solution at 95°C for 5-10 minutes in a thermocycler.
- 11.3.6 Place the PCR tubes at room temperature. If liquid is collected on the inside of the caps, briefly spin the liquid down to the bottom. DNA is now ready for use or storage at -20°C.

12. ANALYSIS

For real time PCR analysis, we recommend the use of 1-2 μ L of eluted DNA in a 20 μ L PCR reaction. If input DNA will be used, it should be diluted 10 fold before adding to the 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 required.

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 0.5-10 µg (about 0.5-1 x 10 ⁶ cells). The minimum amount of chromatin 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

RESOURCES

	Inappropriate DNA fragmenting condition	<p>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.</p> <p>If using a probe-based sonicator, shearing conditions should also be optimized to allow DNA fragment size to be between 200-1000 bp</p>
	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	<p>Ensure the PCR is properly programmed.</p> <p>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.</p>

RESOURCES

	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	<p>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:</p> <ol style="list-style-type: none"> 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.

RESOURCES

	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 (i.e. 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

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria

Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France

Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany

Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain

Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland

Email: technical@abcam.com

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America

Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada

Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific

Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan

Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp