ab117137 – ChIP Kit – Plants

Instructions for Use

For carrying out a successful chromatin immunoprecipitation from plant cells

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

Version 4 Last Updated 29 January 2019

Table of Contents

1. BACKGROUND

Protein-DNA interactions play a critical role for cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, and epigenetic silencing. In plants, interactions between the DNA-binding proteins and cognate promoter sequences are primary determinants in establishing spatial and temporal expression patterns of genes that effect homeostasis, development, and adaptation.

Chromatin Immunoprecipitation (ChIP) offers an advantageous tool for identifying direct genome-wide associations between specific regulatory proteins and their target genes. Unlike other methods such as EMASA, DNA microarrays, and report gene assays, which analyze direct interactions between protein and DNA in vitro, ChIP can detect that a specific protein binds to the specific sequences of a gene in living cells.

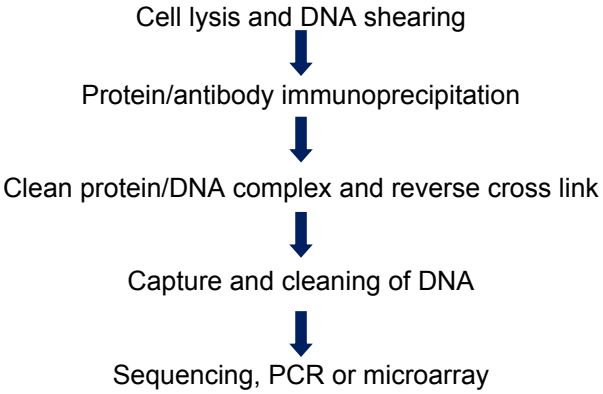
There are several methods used for chromatin immunoprecipitation, however most of these methods available so far are considerably time consuming, labor intensive, or have low throughput. ab117137 use a proprietary and unique procedure and composition to investigate protein-DNA interaction in vivo efficiently.

Abcam's ChIP Kit series have the following features:

- The fastest procedure currently available, which can be finished within 6 hours
- Strip microplate format makes the assay flexible: manual or high throughput
- Columns for DNA purification are included: save time and reduce labor
- Compatible with all DNA amplification-based approaches
- Simple, reliable, and consistent assay conditions

Abcam's ChIP Kit – Plants - contains all reagents required for carrying out a successful chromatin immunoprecipitation from plant cells. Particularly, this kit includes a ChIP-grade dimethyl-histone H3K9 antibody and a negative control normal mouse IgG. Chromatin from the cells is extracted, sheared, and added into the microwell immobilized with the antibody. DNA is released from the antibody-captured protein-DNA complex, reversed, and purified through the specifically designed Fast-Spin Column. Eluted DNA can be used for various down-stream applications.

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 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.

5. MATERIALS SUPPLIED

Item	24 Tests	48 Tests	Storage Condition (Before Preparation)
Wash Buffer	28 mL	2 x 28 mL	RT
Antibody Buffer	15 mL	30 mL	RT
5X Lysis Buffer I	12 mL	24 mL	RT
Lysis Buffer II	3 mL	6 mL	RT
Lysis Buffer III	2 mL	4 mL	RT
Lysis Buffer IV	1.5 mL	5 mL	RT
ChIP Dilution Buffer	2 mL	1 x 6 mL	RT
DNA Release Buffer	2 mL	2 x 2 mL	RT
Reverse Buffer	2 mL	2 x 2 mL	RT
Binding Buffer	5 mL	8 mL	RT
Elution Buffer	0.6 mL	1.2 mL	RT
100X Protease Inhibitor Cocktail	25 µL	50 µL	4°C
Normal Mouse IgG (1 mg/mL)	10 µL	10 µL	4°C
Anti-H3K9me2 (1 mg/mL)	5 µL	8 µL	4°C
Proteinase K (10 mg/mL)	25 µL	50 µL	4°C
8-Well Assay Strips (with frame)	3	6	4°C
8-Well Strip Caps	3	6	RT
F-Spin Column	30	50	RT
F-Collection Tube	30	50	RT

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
- Vortex mixer
- Centrifuge (up to 14,000 rpm)
- Dounce homogenizer
- Sonicator
- Orbital shaker
- Pipettes and pipette tips
- 1.5 mL microcentrifuge tubes
- 15 mL conical tube
- 50 mL Falcon tube
- 37% Formaldehyde
- 2 M Glycine solution
- 70% and 90% Ethanol
- 1X TE Buffer (pH 8.0)
- 14.3 M β-mercaptoethanol (BME)
- Antibody of interest

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

All reagents provided are ready to use.

10. SAMPLE PREPARATION

This kit is designed for 24 or 48 ChIP reactions, not for 24 or 48 samples. The standard protocol of the kit allows for performing 8 reactions with one sample. For testing more samples, the amount of each sample should be reduced. The amount of each reagent used for chromatin preparation should be also proportionally reduced.

10.1 Tissue Collection and In Vivo Cross-Link

10.1.1 Harvest 0.8-1 g of plant tissue (flowers, leaves, or young seedlings) after growth on soil or in vitro in a 50 mL Falcon tube.

10.1.2 Rinse tissue gently with 20 mL of deionized water two times. Remove as much water as possible from the tissue and add 20 ml of 1.0% formaldehyde.

10.1.3 Stuff the top of the 50 mL conical tube (containing the formaldehyde soaked tissue) with nylon mesh to keep the tissue immersed during vacuum infiltration (and aid later rinse steps). Also poke needle-sized holes in the cap of the conical tube and screw on the cap.

10.1.4 Vacuum infiltrate the tissue for 10 minutes in a desiccator attached to a vacuum pump. The formaldehyde solution should boil.

10.2 Tissue Lysis and DNA Shearing

10.2.1 Quench cross-linking by adding 1.25 mL of 2 M Glycine solution (final concentration 0.125 M) and continue vacuum infiltration for an additional 5 minutes.

10.2.2 Remove the formaldehyde and rinse the tissue two times with 20 mL of deionized water. After the rinses, remove as much water as possible (at this stage the cross-linked tissue can either be frozen in liquid nitrogen and stored at -80°C, or used directly for chromatin extraction).

10.2.3 Dilute 5X Lysis Buffer I with distilled water at a 1:5 ratio (1X Lysis Buffer I). Add 3.5 µL of BME to each 10 mL of 1X Lysis Buffer I. Grind the tissue in liquid nitrogen to a fine powder. Add the powder to 20 mL of cold 1X Lysis Buffer I containing BME in a 50 mL conical tube, then vortex, and place on ice.

10.2.4 Filter solution through two layers of Miracloth into a 50 mL tube and centrifuge the filtered solution at 4000 rpm (1900X g) for 20 minutes.

10.2.5 Add 1 µL of BME into each 1 mL of Lysis Buffer II. Remove supernatant and re-suspend pellet in 1 mL of Lysis Buffer II containing BME. Transfer the re-suspended pellet to a 1.5 mL vial and centrifuge at 12,000 rpm for 10 minutes at 4°C to pellet nuclei (white pellet should be seen at this stage).

10.2.6 Add 1 µL of BME into each 1 mL of Lysis Buffer III. Remove supernatant and re-suspend pellet in 300 µL of Lysis Buffer III containing BME.

10.2.7 Add 300 µL of Lysis Buffer III containing BME into a new 1.5 mL vial. Layer the re-suspended pellet from step 6 on top of this 300 µL cushion and centrifuge at 14,000 rpm for 45 minutes at 4°C.

10.2.8 Remove supernatant and re-suspend chromatin pellet in 500 µL of Lysis Buffer IV containing Protease Inhibitor Cocktail (PIC) (e.g. 10 µL of PIC to each 1 mL of Lysis Buffer IV). Shear DNA by sonication. For example, sonicate chromatin solution on ice five times, 15 seconds each at 40%

duty cycle; power setting 2). Place the sample on ice for 1 minute between each sonication treatment. (The conditions of cross-linked DNA shearing can be optimized based on cells and sonicator equipment. If desired, remove 5 µL of sonicated cell lysate for agarose gel analysis. The length of sheared DNA should be between 200-1000 bp).

10.2.9 Pellet cell debris by centrifuging at 14,000 rpm for 10 minutes at 4°C.

11. ASSAY PROCEDURE

11.1 Antibody Binding to the Assay Plate

11.1.1 Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Wash strip wells once with 150 µL of Wash Buffer.

11.1.2 Add 100 µL of the Antibody Buffer to each well and then add the antibodies: 1 µL of Normal Mouse IgG as the negative control, 1 µL of Anti-H3K9me2 as the positive control, and 2-3 µg of an antibody of interest.

11.1.3 Cover the strip wells with Parafilm M and incubate at room temperature for 60-90 minutes. After incubation, remove the incubated antibody solution and wash the strip wells three times with 150 µL of the Antibody Buffer by pipetting in and out. (During incubation time, the cell extracts can be prepared as described in the next steps).

11.2 Protein/DNA Immunoprecipitation

11.2.1 Transfer clear supernatant to a new 1.5 mL vial. (Supernatant can be stored at -80°C at this step.) Dilute the required volume of supernatant with ChIP Dilution Buffer at a 1:1 ratio (e.g. add 100 µL of ChIP Dilution Buffer to 100 µL of supernatant).

11.2.2 Remove 5 µL of the diluted supernatant to a 0.5 mL vial. Label the vial as “input DNA” and then place on ice.

11.2.3 Transfer 100 µL of the diluted supernatant to each antibody-bound strip well. Cover the strip wells with Parafilm M and incubate at room temperature (22-25°C) for 60-90 minutes on an orbital shaker (50-100 rpm).

11.2.4 Remove supernatant. Wash the wells six times with 150 µL of the Wash Buffer. Allow 2 minutes on a rocking platform (100 rpm) for each wash. Wash the wells once (for 2 minutes) with 150 µL of 1X TE Buffer.

11.3 **Cross-Linked DNA Reversal/DNA Purification**

11.3.1 Add 1 µL of Proteinase K to each 40 µL of the DNA Release Buffer and mix. Add 40 µL of the DNA Release Buffer containing Proteinase K to the samples (including the "input DNA" vial). Cover the sample wells with strip caps and incubate at 65°C in a waterbath for 15 minutes.

11.3.2 Add 40 µL of the Reverse Buffer to the samples; mix, and re-cover the wells with strip caps and incubate at 65°C in a waterbath for 90 minutes. Also add 40 µL of the Reverse Buffer to the vial containing supernatant, labeled as "input DNA". Mix and incubate at 65°C for 90 minutes.

11.3.3 Place a spin column into a 2 mL collection tube. Add 150 µL of the Binding Buffer to the samples and transfer mixed solution to the column. Centrifuge at 12,000 rpm for 20 seconds.

11.3.4 Add 200 µL of 70% ethanol to the column, centrifuge at 12,000 rpm for 15 seconds. Remove the column from the collection tube and discard the flowthrough.

11.3.5 Replace column to the collection tube. Add 200 µL of 90% ethanol to the column and centrifuge at 12,000 rpm for 20 seconds.

11.3.6 Remove the column and discard the flowthrough. Replace the column to the collection tube and wash the column again with 200 µL of 90% ethanol at 12,000 rpm for 35 seconds.

11.3.7 Place the column in a new 1.5 mL vial. Add 10-20 µL of Elution Buffer directly to the filter in the column and centrifuge at 12,000 rpm for 20 seconds to elute purified DNA.

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

12. ANALYSIS

For PCR positive control, the primers for At4g03770 or At4g03800 could be used, which represent retrotransposons located within the heterochromatic knob on chromosome 4 of Arabidopsis thaliana and are associated with di-methylated H3K9. For conventional 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 lysate condition (fresh or frozen).

13. TROUBLESHOOTING

Problem	Cause	Solution
Little or No PCR Products	Insufficient tissues	Increase tissue amount (e.g. >10 mg tissues/per reaction)
	Insufficient or too much cross-linking	Check if the appropriate cross-link step is carried out according to the protocol
	Insufficient cell lysis	Follow the guidelines in the protocol. Check the cell lysis by observing a 5 µL portion of the tissue lysate under the microscope
	Insufficient/too much sonication	Follow the protocol instruction for obtaining the appropriate sized DNA. Keep the sample on ice during the sonication
	Incorrect temperature or insufficient time for DNA release and reversal of cross-linking	Follow the guidelines in the protocol for appropriate temperature and time
	Incorrect PCR conditions	Check if all PCR components are added. Increase the amount of DNA added to PCR reaction. Increase the number of cycles for PCR reaction

	Incorrect or bad primers	Ensure the designed primers are specific to the target sequence
	The column is not washed with 90% ethanol	Ensure that wash solution is 90% ethanol
	DNA is not completely passed through the filter	Purify DNA before modification and increase centrifuge time to 1 minute at steps 11.3.3-11.3.7
Little or No Amplification Difference Between the Sample and the Negative Control	Insufficient wash at each wash step.	Follow the protocol for appropriate wash
	Antibody is added into the well for the negative control by mistake	Ensure antibody is added into the correct well
	Too many PCR cycles	If using conventional PCR, decrease the cycles to appropriate cycle number. Differences between quantities of starting DNA can be measured generally within the linear PCR amplification phase
	Little or no enrichment of the target protein in target promoters	N/A

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