Cold Spring Harbor Protocols

EpiSeeker ChIP Kit - Plants

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EpiSeeker 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.

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ab117137 EpiSeeker ChIP Kit - Plants

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1. Overview

The EpiSeeker 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 H3-K9 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. 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.

The EpiSeeker 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.

3. Components and Storage

A. Kit Components

Item	Quantity
ECP1 (Wash Buffer)	28 mL
ECP2 (Antibody Buffer)	15 mL
ECP3C (5X Lysis Buffer I)	12 mL
ECP3D (Lysis Buffer II)	2 x 1.5 mL
ECP3E (Lysis Buffer III)	2 mL
ECP3F (Lysis Buffer IV)	1.5 mL
ECP4 (ChIP Dilution Buffer)	2 mL
ECP5 (DNA Release Buffer)	1 mL
ECP6 (Reverse Buffer)	1 mL
ECP7 (Binding Buffer)	5 mL
ECP8 (Elution Buffer)	0.6 mL
Protease Inhibitor Cocktail (100X)*	25 µL
Normal Mouse IgG (1 mg/ml)*	10 µL

Anti-Dimethyl H3-K9 (1 mg/ml)*	5 µL
Proteinase K (10 mg/ml)*	25 µL
8-Well Assay Strips (with frame)	3
8-Well Strip Caps	3
F-Spin Column	30
F-Collection Tube	30

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

B. Additional Materials Required

- Variable temperature waterbath
- Vortex mixer
- Desktop 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
- 100 mm plate

- Formaldehyde
- Glycine
- β-mercaptoethanol
- Antibody of interest
- TE buffer (pH 8.0)
- Ethanol (96-100%)

C. Storage

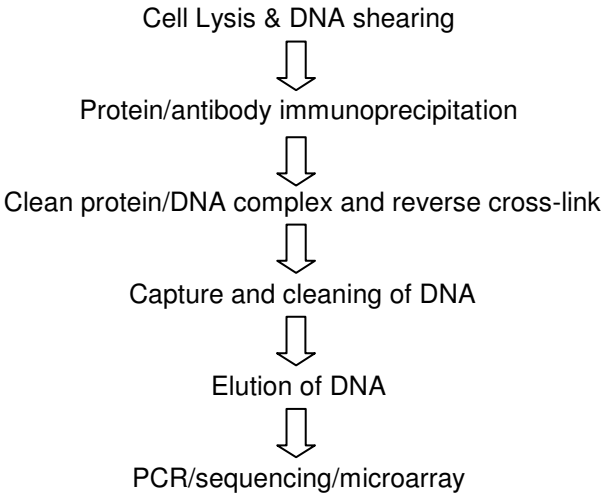
- Store the following components at 4℃: Protease Inhibitor Cocktail, Normal Mouse IgG, Proteinase K, Anti-Dimethyl H3-K9 and 8-Well Assay Strips.

- Store all other components at room temperature.

  The kit is stable for up to 6 months from the shipment date, when stored properly.

4. Protocol

Protocol Summary



Before starting, perform the following:

1. Prepare the following required solutions (not included): 90% Ethanol; 70% Ethanol; 37% Formaldehyde; 2 M Glycine Solution; 14.3 M β-mercaptoethanol (BME); 1X TE Buffer (pH 8.0).
2. Ensure that all buffers are in clear solution. Shake or vortex if these buffers precipitate.

Antibody Binding to the Assay Plate

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 ECP1.
2. Add 100 µl of ECP2 to each well and then add the antibodies: 1 µl of Normal Mouse IgG as the negative control, 1 µl of Anti-Dimethyl H3-K9 as the positive control, and 2-3 µg of an antibody of interest.
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 ECP2 by pipetting in and out. (During incubation time, the cell extracts can be prepared as described in the next steps.)

Tissue Collection and In Vivo Cross-Link

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

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

Tissue Lysis and DNA Shearing

1. Quench cross-linking by adding 1.25 ml of 2M Glycine solution (final concentration 0.125M) and continue vacuum infiltration for an additional 5 minutes.
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).
3. Dilute ECP3C with distilled water at a 1:5 ratio (1X ECP3C). Add 3.5 µl of BME to each 10 ml of 1X ECP3C. Grind the tissue in liquid nitrogen to a fine powder. Add the powder to 20 ml of cold 1X ECP3C in a 50 ml conical tube, then vortex, and place on ice.

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.
5. Add 1 µl of BME into each 1 ml of ECP3D. Remove supernatant and re-suspend pellet in 1 ml of ECP3D 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).
6. Add 1 µl of BME into each 1 ml of ECP3E. Remove supernatant and re-suspend pellet in 300 µl of ECP3E containing BME.
7. Add 300 µl of ECP3E 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.
8. Remove supernatant and re-suspend chromatin pellet in 500 µl of ECP3F containing Protease Inhibitor Cocktail (PIC) (ex: 10 µl of PIC to each 1 ml of ECP3F). 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.)

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

Protein/DNA Immunoprecipitation

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 ECP4 at a 1:1 ratio (ex: add 100 µl of ECP4 to 100 µl of supernatant).
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.
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).
4. Remove supernatant. Wash the wells six times with 150 µl of ECP1. 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.

Cross-Linked DNA Reversal/DNA Purification

1. Add 1 µl of Proteinase K to each 40 µl of ECP5 and mix. Add 40 µl of ECP5 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.
2. Add 40 µl of ECP6 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 ECP6 to the vial containing supernatant, labeled as "input DNA". Mix and incubate at 65°C for 90 minutes.
3. Place a spin column into a 2 ml collection tube. Add 150 µl of ECP7 to the samples and transfer mixed solution to the column. Centrifuge at 12,000 rpm for 20 seconds.
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.
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.
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.

7. Place the column in a new 1.5 ml vial. Add 10-20 µl of ECP8 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.

Note: 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 H3-K9. 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).

5. Troubleshooting

Problem	Cause	Solution
Little or No PCR Products	Insufficient tissues.	Increase tissue amount (ex: >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.

Problem	Cause	Solution
	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 3-7 of "Cross-Linked DNA Reversal/DNA Purification."
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.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

6. Related Products

- EpiSeeker ChIP Kit - Histone H3 (methyl K9) (ab117141)
- EpiSeeker ChIP Kit - Histone H3 (methyl K4) (ab117143)
- EpiSeeker ChIP Kit - Histone H3 (acetyl) (ab117147)
- EpiSeeker ChIP Kit - Histone H4 (acetyl) (ab117148)

Abcam in the USA
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

Abcam in Japan
Abcam KK
2-2-1 Nihonbashi
Horidome-cho,
Chuo-ku, Tokyo
103-0012
Japan

Tel: +81-(0)3-6231-094
Fax: +81-(0)3-6231-0941

Abcam in Europe
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

Abcam in Hong Kong
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682
Fax: (852) 3016-1888