ab156906 – Plant Chromatin Extraction Kit

Instructions for Use

For isolating chromatin or DNA-protein complex from plants in a simple and rapid format

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

Version 1 Last Updated 6 February 2019

Table of Contents

1. BACKGROUND

Chromatin immunoprecipitation (ChIP) offers an advantageous tool for studying protein-DNA interaction. With ChIP, the experimenter can determine if a specific protein binds to specific sequences of a gene in living cells by combining it with PCR (ChIP-PCR), microarray (ChIP-chip), or sequencing (ChIP-Seq) techniques. When performing ChIP, chromatin or DNA-protein complex in plant tissues need to be first isolated in an efficient manner. However, the existing methods used for plant chromatin preparation are inconvenient and time consuming.

Abcam's Plant Chromatin Extraction Kit (ab156906) addresses these issues by introducing the following features:

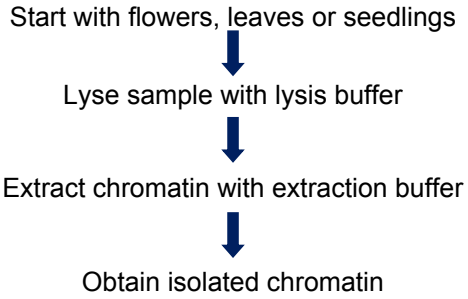
- Extremely fast procedure: the entire procedure from cell/tissue sample to ready-to-use chromatin is less than 2 hours
- Convenient and flexible: the kit is suitable for preparing both native chromatin and cross-linked chromatin from monolayer or suspension cells, or from tissues
- Unsheared chromatin makes it customizable for various analysis workflows that require either intact or fragmented chromatin, including ChIP, in vitro protein-DNA interaction analysis, nuclear enzyme assay, etc.

Abcam's Plant Chromatin Extraction Kit is suitable for isolating chromatin or DNA-protein complex from plants in a simple and rapid format. Chromatin prepared by using this kit can be used in a variety of chromatin immunoprecipitation methods. It is the optimal method for preparing chromatin required by Abcam's one-hour ChIP procedure using Abcam's ChIP Kit – Plants (ab117137). The isolated chromatin can also be used in other chromatin-related applications such as in vitro protein-DNA binding assays or nuclear enzyme assays.

Abcam's Plant Chromatin Extraction Kit (ab156909) contains all the reagents required for carrying out a successful chromatin extraction directly

from plant tissues. Cell membranes of the sample, with or without cross-linking, are broken down using the provided lysis buffer. Chromatin or DNA-protein complex is then extracted with the extraction buffer. The extracted chromatin can then be diluted with chromatin buffer and stored at the appropriate temperature.

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.

5. MATERIALS SUPPLIED

Item	50 Tests	Storage Condition (Before Preparation)
5X Lysis Buffer	2 x 20 mL	RT
Extraction Buffer A	15 mL	RT
Extraction Buffer B	15 mL	RT
Extraction Buffer C	8 mL	RT
Chromatin Buffer	8 mL	RT
1000X Protease Inhibitor Cocktail	15 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:

- Sonicator
- Vortex mixer
- Centrifuge including desktop centrifuge (up to 14,000 rpm)
- Pipettes and pipette tips
- 1.5 mL microcentrifuge tubes
- 15 mL conical tube
- 50 mL Falcon tubes
- Cells or tissues
- Cell culture medium
- β-mercaptoethanol (BME)
- 1X PBS
- Distilled water
- Miracloth or similar filtration material

If cross-linking chromatin:

- 37% formaldehyde
- 2 M glycine solution
- Vacuum dessicator
- Nylon mesh

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

Prepare fresh reagents immediately prior to use.

9.1 **Working Lysis Buffer**

Add 1 mL of 5X Lysis Buffer to every 4 mL of distilled water. Then add 4 µL of BME to every 10 mL of diluted Lysis Buffer (1X).

9.2 **Working Extraction Buffer A**

Add 1 µL of BME to every 1 mL of Extraction Buffer A.

9.3 **Working Extraction Buffer B**

Add 1 µL of BME to every 1 mL of Extraction Buffer B.

9.4 **Working Extraction Buffer C**

Add 1 µL of 1000X Protease Inhibitor Cocktail for every 1 mL of Extraction Buffer C.

Note: *Put all working buffers on ice before use.*

10. SAMPLE PREPARATION

Input Amount: Harvest 0.1-1 g of plant tissue (flowers, leaves, or young seedlings) after growth in soil or *in vitro* in a 50 mL Falcon tube. The amount of tissue for each preparation can be 100 mg to 1 g. A total of 50 standard extractions can be performed with this kit (use 200 mg of tissue per extraction).

Expected Yield: Yield of chromatin is approximately 0.5-2 µg per 200 mg of tissue, depending on the tissue type.

11. ASSAY PROCEDURE

11.1 Cell Collection and Cross-Linking

Note: *For tissues that are not cross-linked, skip this section and go directly to step 11.2.*

11.1.1 Gently rinse tissue with 20 mL of deionized water, 2 times. Remove as much water as possible from the tissue. Place the tissue into a 50 mL conical tube. Then add 5 mL of 1.0% formaldehyde solution per 200 mg of tissue into the 50 mL conical tube.

11.1.2 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 to aid later rinse steps). Then poke several needle sized holes in the cap of the conical tube and screw the cap on.

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

11.1.4 Quench cross-linking by adding 0.3 mL of 2 M glycine per 5 mL of 1.0% formaldehyde solution (final glycine concentration is 0.125 M). Then continue vacuum infiltration for an additional 5 minutes.

11.1.5 Remove the formaldehyde and rinse the tissue 2 times with 20 mL of deionized water. After the rinses, remove as much water as possible (at this stage the cross-linked tissue can be either frozen in liquid nitrogen and stored at -80°C or used directly for chromatin extraction).

11.2 Tissue Lysis and Chromatin Extraction

11.2.1 Grind the tissue in liquid nitrogen to a fine powder. Pour the powder into a 50 mL conical tube and add 4 mL of cold Working Lysis Buffer (1X) per 200 mg of tissue. Vortex and place on ice.

11.2.2 Filter the solution through 2 layers of Miracloth into a 50 mL conical tube and centrifuge the filtered solution at 4000 rpm (1900X G) for 20 minutes.

11.2.3 Remove supernatant and re-suspend the pellet in 0.3 mL of Working Extraction Buffer A per 200 mg of tissues. Transfer the resuspended pellet to 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).

11.2.4 Remove supernatant and re-suspend the pellet in 100 µL of Working Extraction Buffer B per 200 mg of tissues.

11.2.5 Add 200 µL of Working Extraction Buffer B, per 200 mg of tissue, into a new 1.5 mL microcentrifuge tube. Layer the re-suspended pellet from step 11.2.4 on top of this 200 µL cushion and centrifuge at 14,000 rpm for 45 minutes at 4°C.

11.2.6 Remove supernatant and re-suspend chromatin pellet in 50 µL of Working Extraction Buffer C per 200 mg of tissue

Note: *(Optional) Sonicate 2 cycles at 20 seconds per cycle to increase chromatin extraction. Allow the sample to cool on ice between sonication pulses for 30 seconds. For example, sonication can be carried out with a microtip attached to Branson 450 sonifier, using the 25% power output setting.*

11.2.7 Centrifuge at 12,000 rpm at 4°C for 10 minutes.

11.2.8 Transfer supernatant to a new vial.

11.2.9 Add Chromatin Buffer at a 1:1 ratio (e.g. add 50 µL of Chromatin Buffer to 50 µL of re-suspended chromatin).

The chromatin solution can be used immediately or stored at -80°C after aliquoting until further use. Avoid repeated freeze/thaw cycles.

12. ANALYSIS

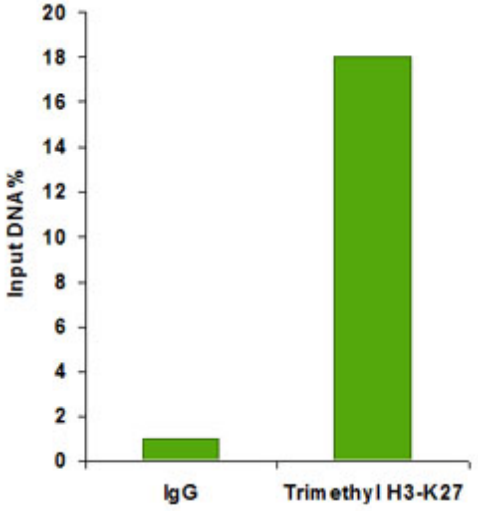


Figure 1. ChIP analysis of H3K27me3 enriched in AGAMOUS gene with chromatin extract prepared from 2-week-old icu2-1/icu2-1 seedlings using Abcam's Plant Chromatin Extraction Kit (ab156906).

13. TROUBLESHOOTING

Problem	Cause	Solution
Low chromatin yield	Insufficient sample size	To obtain the best results the sample size per reaction should be between 100-200 mg of tissue
	Insufficient chromatin extraction	(a) Check that all of the reagents have been added in the correct volumes and in the correct order based on the sample amount; (b) check sample lysis under microscope after addition of lysis buffer; and (c) check if the plant tissue type is compatible with this extraction procedure
	Use of expired lysis or extraction reagents	Check the expiration date of the kit. Expired reagents may cause an inefficient extraction
	Incorrect temperature and/or insufficient time during extraction	Ensure the incubation times and temperatures described in the protocol are followed correctly
Chromatin degradation	Improper sample storage	Chromatin sample should be stored at -80°C (3-6 months). Avoid repeated freeze/thawing cycles

14. NOTES

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