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ChromaFlash™ Plant Chromatin Extraction Kit

Base Catalog # P-2022

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The ChromaFlash™ 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 Epigentek's one-hour ChIP procedure using the ChromaFlash™ One-Step ChIP Kit (P-2025) or the ChromaFlash™ Magnetic ChIP Kit (P-2026). The isolated chromatin can also be used in other chromatin-related applications such as in vitro protein-DNA binding assays or nuclear enzyme assays.

Starting Material and Input amount: Starting materials can include various types of plant tissue, such as flowers, leaves, and young seedlings. 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). Yield of chromatin is approximately 0.5 to 2 µg per 200 mg of tissue, depending on the tissue type.

Precautions: 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.

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KIT CONTENTS

Component	50 Preparations P-2022-050	Storage Upon Receipt
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
Protease Inhibitor Cocktail (1000X)*	15 µl	4°C
User Guide	1	RT

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store Protease Inhibitor Cocktails at 4°C; (2) Store remaining components at room temperature.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ 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 conical tubes
- ☐ Cells or tissues
- ☐ Cell culture medium
- ☐ β-mercaptoethanol (BME)
- ☐ 1X PBS
- ☐ Distilled water

- ☐ Miracloth or similar filtration material
- ☐ 37% formaldehyde (for optional cross linking step)
- ☐ 2 M glycine solution (for optional cross linking step)
- ☐ Vacuum dessicator (for optional cross linking step)
- ☐ Nylon mesh (for optional cross linking step)

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the ChromaFlash™ Plant Chromatin Extraction Kit is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The ChromaFlash™ Plant Chromatin Extraction Kit is for research use only and is not intended for diagnostic or therapeutic application.

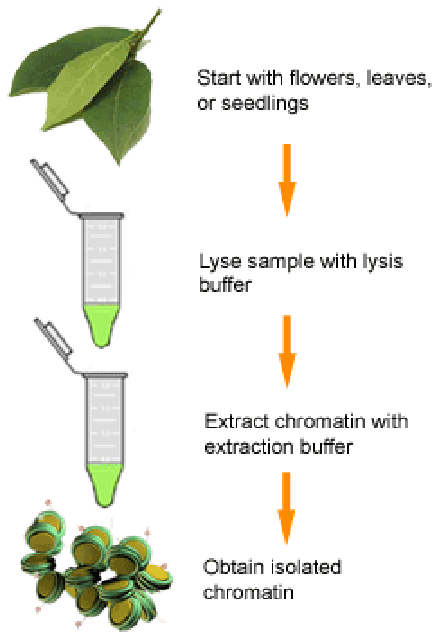
A BRIEF OVERVIEW

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. The ChromaFlash™ Plant Chromatin Extraction Kit addresses these issues by introducing the following features:

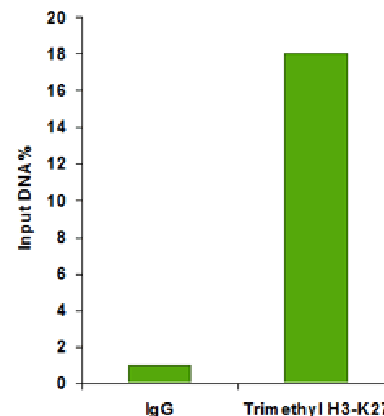
- Extremely fast procedure: the entire procedure from cell/tissue sample to ready-to-use chromatin is less than 2 hours.
- Convenient and flexible: the kit is suitable for preparing both native chromatin and cross-linked chromatin from monolayer or suspension cells, or from tissues.
- Unsheared chromatin 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.

PRINCIPLE & PROCEDURE

The ChromaFlash™ Plant Chromatin Extraction Kit 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.



Schematic procedure of the ChromaFlash™ Plant Chromatin Extraction Kit.



ChIP analysis of tri-methyl H3-K27 enriched in AGAMOUS gene with chromatin extract prepared from 2-week-old icu2-1/icu2-1 seedlings using the ChromaFlash™ Plant Chromatin Extraction Kit.

Assay Protocol

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: Harvest 0.2 to 1 g of plant tissue (flowers, leaves, or young seedlings) after growth in soil or *in vitro* in a 50 ml Falcon tube.

Expected Yield: 5 preparations can be performed when 1 g of tissue is used (0.2 g per extraction).

1. Preparation of Working Buffers and Solutions

a. Prepare **Working Lysis Buffer** by adding 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 **Working Lysis Buffer** (1X).

b. Prepare **Working Extraction Buffer A** by adding 1 µl of BME to every 1 ml of **Extraction Buffer A**.

c. Prepare **Working Extraction Buffer B** by adding 1 µl of BME to every 1 ml of **Extraction Buffer B**.

d. Prepare **Working Extraction Buffer C** by adding 1 µl of **Protease Inhibitor Cocktail** for every 1 ml of **Extraction Buffer C**.

Note: Put all working buffers on ice before use.

2. Cell Collection and Cross-Linking

Note: For tissues that are not cross-linked, skip this section and go directly to step 3a of "Tissue Lysis and Chromatin Extraction."

a. 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.

b. 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.

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

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

e. 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).

3. Tissue Lysis and Chromatin Extraction

a. 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.

b. 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.

c. 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).

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

e. 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 3d on top of this 200 µl cushion and centrifuge at 14,000 rpm for 45 min at 4°C.

f. 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.

g. Centrifuge at 12,000 rpm at 4°C for 10 min.

h. Transfer supernatant to a new vial.

i. Add **Chromatin Buffer** at a 1:1 ratio (e.g., add 50 µl of **Chromatin Buffer** to 50 µl of re-suspended chromatin).

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

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low chromatin yield	Insufficient sample size.	To obtain the best results the sample size per ChIP 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, which is 6 months from the time of shipment. 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.

RELATED PRODUCTS

Chromatin Shearing and Cleanup
P-1006 DNA Concentrator Kit

Sonication Instruments
EQC-2000 EpiSonic™ 2000 Sonication System

ChIP Reaction
P-2025 ChromaFlash™ One-Step ChIP Kit
P-2026 ChromaFlash™ Magnetic ChIP kit

PCR Analysis
P-1029 EpiQuik Quantitative PCR Fast Kit