

ChromaFlash™ Chromatin Isolation & Shearing Kit

Base Catalog # P-2023

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The ChromaFlash™ Chromatin Isolation & Shearing Kit is suitable for isolating and shearing chromatin from mammalian cells or tissues in a simple and rapid format. Chromatin sheared with this kit can be used in a variety of chromatin immunoprecipitation methods. The sheared chromatin can also be used in other chromatin-related applications such as in vitro protein-DNA binding assays.

Starting Material and Input amount: Starting materials can include various tissue or cell samples such as cells from flask or microplate cultured cells, fresh and frozen tissues, etc. The amount of cells and tissues for each preparation can be 2×10^5 to 1×10^7 cells and 10 mg to 400 mg, respectively. For optimal preparation, the input amount should be 2 to 5×10^6 cells or 100 to 200 mg tissues. A total of 100 standard extractions (use 2×10^6 cells or 100 mg of tissue per extraction) can be performed with this kit. The yield of sheared chromatin is approximately $3 \mu\text{g}$ per 10^6 cells or per 50 mg tissues.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube/vials. 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.

KIT CONTENTS

Component	100 Preparations P-2023-100	Storage Upon Receipt
10X Lysis Buffer	11 ml	RT
Extraction Buffer	22 ml	RT
Chromatin Buffer	22 ml	RT
Protease Inhibitor Cocktails (1000X)*	110 µl	4°C
User Guide	1	RT

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

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt: (1) 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.

Note: Check if any buffers contain salt precipitates before use. If so, shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Sonicator
- Vortex mixer
- Dounce homogenizer
- Centrifuge including desktop centrifuge (up to 14,000 rpm)
- Pipettes and pipette tips
- 1.5 ml microcentrifuge tubes
- 15 ml conical tube
- Cells or tissues
- Cell culture medium
- 37% formaldehyde (if cross-linked)
- 1.25 M Glycine solution (if cross-linked)
- 1X PBS

- Distilled water

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the ChromaFlash™ Chromatin Isolation & Shearing 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™ Chromatin Isolation & Shearing 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. The experimenter can determine if a specific protein binds to the specific sequences of a gene in living cells by combining ChIP with PCR (ChIP-PCR), microarray (ChIP-chip), or sequencing (ChIP-Seq) techniques. For example, the measurement of the amount of methylated histone H3 at lysine 9 (meH3-K9) associated with a specific gene promoter region under various conditions can be achieved through a ChIP-PCR assay, while recruitment of meH3-K9 to the promoters on a genome-wide scale can be detected by ChIP-chip. In particular, the ChIP method with specific antibodies directly against various transcriptional factors is widely demanded.

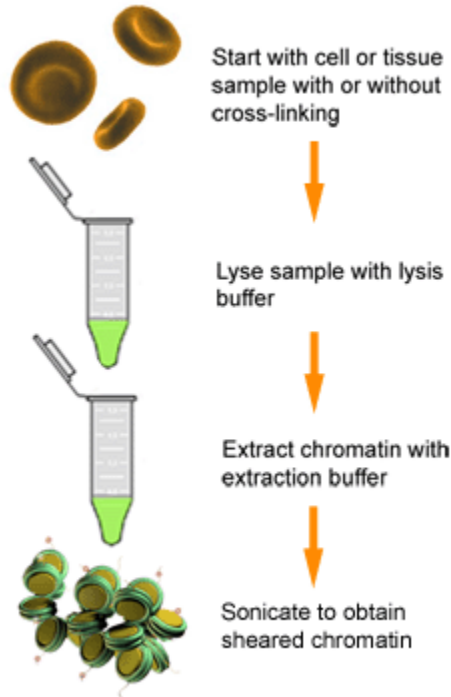
For performing ChIP, chromatin or DNA-protein complex in cells or tissues should first be isolated and sheared to desired size. The ChromaFlash™ Chromatin Isolation & Shearing Kit addresses the inconvenience and time consuming issues of existing chromatin preparation methods by introducing the following features:

- Fast procedure: the entire procedure from cell/tissue sample to ready-to-use sheared 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.
- Chromatin shearing is compatible with various sonication platforms including waterbath-based, probe-based sonicators and enzyme-based methods.

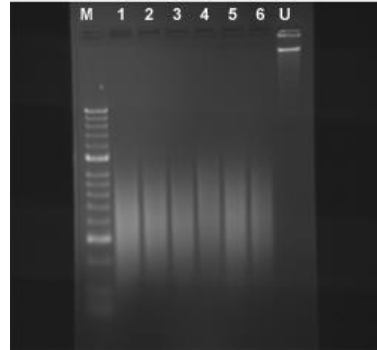
PRINCIPLE & PROCEDURE

The ChromaFlash™ Chromatin Isolation & Shearing Kit contains all reagents required for carrying out successful chromatin extraction and shearing directly from mammalian cells or tissues. Cell

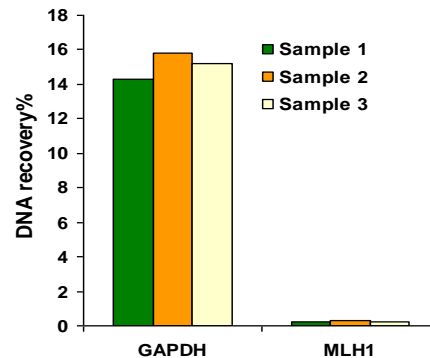
membranes of the sample, with or without cross-linking, are broken down using the provided lysis buffer. Chromatin or DNA-protein complex is then extracted with the extraction buffer. The extracted chromatin can then be sheared and stored at the appropriate temperature after being diluted in chromatin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



Schematic procedure of the ChromaFlash™ Chromatin Isolation/Shearing Kit



Chromatin was extracted from six MDA-231 cell samples. 40 ul (8 ug) of chromatin were sheared in a 0.2 ml tube using the EpiSonic-1100 for 20 cycles (15 sec On and 30 sec Off). DNA was purified and then run on a gel.



The sheared chromatin was used for ChIP-qPCR analysis of RNA polymerase II enrichment in GAPDH and MLH1 promoters by using the ChromaFlash One-Step ChIP Kit (P-2025) and the Methylamp™ MS-qPCR Fast Kit (P-1028).

Assay Protocol

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

Starting Materials

Monolayer cells: 2×10^5 to 1×10^7 cells per preparation.

Suspension cells: 4×10^5 to 2×10^7 cells per preparation.

Tissues: 10 mg to 400 mg per preparation.

1. Preparation of Working Buffers and Solutions

- a. Prepare **Working Lysis Buffer** by adding 1 ml of **10X Lysis Buffer** and 6 μ l of **Protease Inhibitor Cocktail** to every 9 ml of distilled water.
- b. Prepare **Working Extraction Buffer** by adding 1 μ l of **Protease Inhibitor Cocktail** to every 1 ml of **Extraction Buffer**

2. Cell Collection and Cross-Linking

For Monolayer or Adherent Cells:

- a. Grow cells (treated or untreated) to 80%-90% confluence on a 100 mm plate (about 2×10^6 to 4×10^6 cells; 2×10^6 cells are required for each standard preparation), then trypsinize and collect them into a 15 ml conical tube. Count the cells in a hemocytometer.
- b. Centrifuge the cells at 1000 rpm for 5 min. Discard the supernatant.
- c. Wash cells with 10 ml of PBS once by centrifugation at 1000 rpm for 5 min. Discard the supernatant.

Note: For cells that are not cross-linked, go directly to Step 3d after Step 2c.

- d. Add 9 ml fresh cell culture medium containing formaldehyde with a final concentration of 1% (i.e., add 270 μ l of 37% formaldehyde to 10 ml of cell culture medium) to cells.
- e. Incubate at room temperature (20-25°C) for 10 min on a rocking platform (50-100 rpm).

For Suspension Cells:

- a. Collect cells (treated or untreated) into a 15 ml conical tube. (2×10^6 to 5×10^6 cells are required for each preparation). Count cells in a hemocytometer.
- b. Centrifuge the cells at 1000 rpm for 5 min. Discard the supernatant.
- c. Wash cells with 10 ml of PBS once by centrifugation at 1000 rpm for 5 min. Discard the supernatant.

Note: For cells that are not cross-linked, go directly to Step 3d after Step 2c.

- d. Add 9 ml fresh cell culture medium containing formaldehyde with a final concentration of 1% (i.e., add 270 μ l of 37% formaldehyde to 10 ml of cell culture medium) to cells.
- e. Incubate at room temperature (20-25°C) for 10 min on a rocking platform (50-100 rpm).

For Tissues:

- a. Put the tissue sample into a 60 or 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample.
- b. Weigh the sample and cut the sample into small pieces ($1-2 \text{ mm}^3$) with a scalpel or scissors.

Note: For tissues that are not cross-linked, go directly to Step 2j after Step 2b.

- c. Transfer tissue pieces to a 15 ml conical tube.

- d. Prepare cross-link solution by adding formaldehyde to cell culture medium with a final concentration of 1%. (e.g., add 270 μ l of 37% formaldehyde to 10 ml of culture medium).
- e. Add 1 ml of cross-link solution for every 50 mg tissues.
- f. Incubate at room temperature for 15-20 min on a rocking platform.
- g. Add 1 ml of 1.25 M glycine for every 9 ml of cross-link solution.
- h. Mix and centrifuge at 800 rpm for 5 min. Discard the supernatant.
- i. Wash cells with 10 ml of ice-cold PBS once by centrifugation at 800 rpm for 5 min. Discard the supernatant.
- j. Transfer tissue pieces to a Dounce homogenizer.
- k. Add 0.5 ml **Working Lysis Buffer** for every 50 mg tissues.
- l. Disaggregate tissue pieces by 20-40 strokes.
- m. Transfer homogenized mixture to a 15 ml conical tube and centrifuge at 3000 rpm for 5 min at 4°C. If total mixture volume is less than 2 ml, transfer mixture to a 2 ml vial and centrifuge at 5000 rpm for 5 min at 4°C. Then go directly to Step 3f.

3. Cell Lysis and Chromatin Extraction

- a. Add 1 ml of 1.25 M glycine for every 9 ml of cross-link solution.
- b. Mix and centrifuge at 1000 rpm for 5 min.
- c. Remove medium and wash cells once with 10 ml of ice-cold PBS by centrifuging at 1000 rpm for 5 min.
- d. Add **Working Lysis Buffer** to re-suspend the cell pellet (200 μ l/ 1×10^6 cells) and incubate on ice for 10 min
- e. Vortex vigorously for 10 sec and centrifuge at 3000 rpm for 5 min.
- f. Carefully remove supernatant.
- g. Add **Working Extraction Buffer** to re-suspend the chromatin pellet (100 μ l/ 1×10^6 cells, 500 μ l maximum for each vial).
- h. Transfer the chromatin lysate to a 1.5 ml vial and incubate on ice for 10 min and vortex occasionally.

4. Chromatin Shearing

- a. Resuspend the chromatin lysate by vortexing.
- b. Shear chromatin with one of the following methods:

Waterbath Sonication:

Epigentek EpiSonic 1100 (Epigentek Cat No. EQC-1100): Use 50 ul of chromatin lysate per 0.2 ml tube or per PCR plate well. Shear 20 cycles, 15 second On, 30 second Off, each at 170-190 watts.

Diagenode Bioruptor: Use 200 ul of chromatin lysate per 1.5 ml vial. Shear 3 runs of 10 cycles, 30 second On, 30 second Off, each at high power setting. Spin and vortex between each run.

Covaris S2 Instrument: Use 130 ul of chromatin lysate per Covaris microTube. Duty cycle: 2%, Intensity: 3, Cycles per burst: 200, Total processing time: 10 min.

Probe-based Sonication:

Use 300 ul of chromatin lysate per 1.5 ml tube. As an example, sonication can be carried out with a microtip attached to a Branson 450 sonifier, set to 25% power output. Sonicate 3-4 pulses of 10-15 seconds each, followed by 30-40 seconds rest on ice between each pulse. (The conditions of cross-linked DNA shearing can be optimized based on cells and sonicator equipment).

If desired, remove 10 µl of sonicated cell lysate for agarose gel analysis along with a DNA marker on a 1 - 2% agarose gel. Stain with Ethidium Bromide or other fluorescent dye for DNA and visualize it under ultraviolet light.

The length of sheared DNA should be between 200-1000 bp.

Note: The isolated chromatin can also be sheared with various enzyme-based methods. The optimization of the shearing conditions, for example enzyme concentration and incubation time, is needed in order to use enzyme-based methods.

- c. Centrifuge at 12,000 rpm at 4°C for 10 min.
- d. Transfer supernatant to a new vial.
- e. Add **Chromatin Buffer** at a 1:1 ratio (e.g., add 100 µl of **Chromatin Buffer** to 100 µl of supernatant).

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

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of chromatin	Insufficient amount of samples.	To obtain the best results, the amount of samples should be 2 to 5 x 10 ⁶ cells or 100 to 200 mg tissues per ChIP reaction.
	Insufficient chromatin extraction.	Ensure that all reagents have been added with the correct volume and in the correct order based on the sample amount.
		Check for sample lysis under microscope after the tissue/cell lysis step.
		Ensure that the cell or tissue species

		are compatible with this extraction procedure.
	Lysis or extraction reagents have expired. Expired reagents may cause inefficient extraction.	Ensure that the kit has not exceeded the expiration date of the kit. Standard shelf life, when stored properly, is 6 months from date of receipt.
	Incorrect temperature and/or insufficient incubation time during extraction.	Ensure the incubation time and temperature described in the protocol are followed correctly.
Degradation of chromatin	Improper storage of chromatin.	Chromatin sample should be stored at -80°C (3-6 months). Avoid multiple freeze/thaw cycles.
There is a cloudy layer in the sheared chromatin solution	Lipid and glycogen are not completely removed during chromatin extraction.	Avoid this layer when supernatant is removed.
Chromatin is not efficiently sheared	Shearing condition is not optimized, especially when probe-based sonicator is used.	To obtain desired DNA size (200-1000 bps), shear chromatin with a time course (ex: 5, 10, 15, 20 pulses) that generates suitably sized DNA for use in ChIP. In general, waterbath based sonicators, such as the EpiSonic-1100, could give consistent and efficient shearing conditions.

RELATED PRODUCTS

Chromatin Shearing and Cleanup

P-1006 DNA Concentrator Kit

Sonication Instruments

EQC-1100 EpiSonic™ Multi-Functional Bioprocessor 1100

ChIP Reaction

P-2025 ChromaFlash™ One-Step ChIP Kit

P-2026 ChromaFlash™ One-Step Magnetic ChIP kit

PCR Analysis

P-1029 EpiQuik™ Quantitative PCR Fast Kit

ChIP-Grade Antibodies

For ChIP-Grade Antibodies, search "chip-grade" at www.epigentek.com

