ab117152 – Chromatin Extraction Kit

Instructions for Use

For the isolation of chromatin or DNA-protein complex from mammalian cells or tissues

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

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1. BACKGROUND

Chromatin immunoprecipitation (ChIP) offers an advantageous tool for studying protein-DNA interaction. With ChIP, researchers can determine if a specific protein binds to the specific sequences of a gene in living cells by combining 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 (H3 methylK9) associated with a specific gene promoter region under various conditions can be achieved through a ChIP-PCR assay, while recruitment of H3 methylK9 to the promoters on a genome-wide scale can be detected by ChIP-chip.

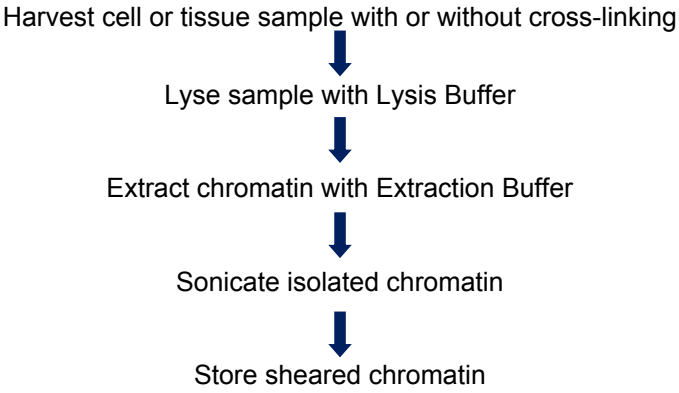
The Chromatin Extraction Kit addresses the inconvenience and time consuming issues of existing chromatin preparation methods by introducing the following features:

- Fast procedure: the entire procedure from cell/tissue sample to ready-to-use chromatin is less than 60 minutes
- Convenient and flexible: this kit is suitable for preparing both native chromatin and cross-linked chromatin from monolayer or suspension cells, or from tissues
- Choose between sheared or un-sheared chromatin: can be used in analyses that require either intact or fragmented chromatin, including ChIP, in vitro protein-DNA interaction analysis or nuclear enzyme assays.

The Chromatin Extraction Kit contains all reagents required for carrying out successful chromatin extraction directly from mammalian cells or tissues. Cell membranes are broken down using the provided lysis buffer and chromatin or DNA-protein complexes are then extracted with the extraction buffer. The extracted chromatin can then be diluted with chromatin buffer and stored at the appropriate temperature.

Chromatin prepared by this kit can be used in a variety of chromatin immunoprecipitation (ChIP) methods. The isolated chromatin can also be used in other chromatin-related applications such as in vitro protein- DNA binding assays and nuclear enzyme assays.

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.

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

5. MATERIALS SUPPLIED

Item	Quantity (100 Tests)	Storage Condition (Before Preparation)
10X Lysis Buffer	11 mL	RT
Extraction Buffer	11 mL	RT
Chromatin Buffer	11 mL	RT
Protease Inhibitor Cocktails (1000X)*	110 µL	4°C

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

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Vortex mixer
- Dounce homogenizer
- Centrifuges, including desktop centrifuge – keep centrifuges at 4°C
- Pipettes and pipette tips
- 1.5 mL microcentrifuge tubes
- 15 mL or 50 mL conical tubes
- Cell culture medium
- 1X PBS
- Distilled water

If cross-linking chromatin:

- 37% formaldehyde
- 1.25 M Glycine solution (previously filtered to sterilize)

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.
- Complete removal of all solutions and buffers during wash steps.

9. REAGENT PREPARATION

Prepare fresh reagents immediately prior to use.

9.1 Working Lysis Buffer (1X)

Prepare Working Lysis Buffer by diluting 10x Lysis Buffer 1/10 and Protease Inhibitor Cocktail 1/1000. For example, to prepare 10 mL Working Lysis Buffer (1X):

Buffer Constituent	Volume
10X Lysis Buffer	**1 mL**
Protease Inhibitor Cocktail (1000x)	**10 µL**
ddH_2O	**9 mL**

9.2 Working Extraction Buffer

Prepare Working Extraction Buffer by adding 1 µL of Protease Inhibitor Cocktail to every 1 mL of Extraction Buffer.

9.3 1% Formaldehyde Culture medium

Add 270 µL of 37% formaldehyde to 10 mL culture medium.

Note: *Keep PBS and all working solutions on ice.*

10. SAMPLE PREPARATION

Starting Materials: Starting materials can include various tissue or cell samples such as cells from flask or plate cultured cells, fresh and frozen tissues, etc.

Input Amount:

- Monolayer cells: $1x10^5$ – $5x10^6$ cells per preparation
- Suspension cells: $2x10^5$ – $1x10^7$ cells per preparation
- Tissues: 10 mg – 200 mg per preparation

Sample Type	Amount	Chromatin Yield
Cells	**1 x 10^5 – 5 x 10^6 cells**	**4 µg/ 10^6 cells**
Tissue	**50 – 200 mg**	**4 µg/ 50 mg**

Considerations when preparing sample material:

- Frozen sample should be thawed on ice. This process could take hours depend on the amount of the pellet; keep that in mind when thinking about experimental timings
- Keep samples on ice at all times to prevent sample degradation by proteases
- 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

11. ASSAY PROCEDURE

11.1 **Protocol for Monolayer or Adherent cells**

11.1.1 Grow cells (treated or untreated) to 80%-90% confluence on a 100 mm plate (about $2x10^6$ – $4x10^6$ cells).

11.1.2 Trypsinize cells as per your usual method and collect them into a 15 mL – 50 mL conical tube. Count the cells in a hemocytometer or Coulter counter.

11.1.3 Centrifuge the cells at 1000 rpm for 5 min. Discard the supernatant.

11.1.4 Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 min. Discard the supernatant. Repeat this step one more time.

***Note**: For cells that are not cross-linked, go directly to Step 11.3.1.*

11.1.5 **For cross-linked cells only:** Add 10 mL of 1% formaldehyde/culture medium solution to the pellet and resuspend by pipetting up and down carefully.

11.1.6 Incubate at room temperature (20-25°C) for 10 min on a rocking platform (50-100 rpm).

11.1.7 Add 1.1 mL of 1.25 M glycine. Mix once by inversion.

11.1.8 Centrifuge cells at 1000 rpm for 5 min. Discard supernatant.

11.1.9 Wash cells by resuspending them in 10 mL of ice-cold PBS in a 15 mL – 50 mL conical tube and centrifuge at 1000 rpm for 5 min. Carefully discard supernatant.

11.2 **Protocol for Suspension cells**

11.2.1 Collect cells (treated or untreated) into a 15 mL – 50 mL conical tube. (1x 10^6 to $2x10^6$ cells are required for each reaction). Count the cells in a hemocytometer or Coulter counter.

11.2.2 Centrifuge the cells at 1000 rpm for 5 min. Discard the supernatant.

11.2.3 Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 min. Discard the supernatant. Repeat this step one more time.

***Note**: For cells that are not cross-linked, go directly to Step 11.3.1.*

11.2.4 **For cross-linked cells only:** Add 10 mL of 1% formaldehyde/culture medium solution to the pellet and resuspend by pipetting up and down carefully.

11.2.5 Incubate at room temperature (20-25°C) for 10 min on a rocking platform (50-100 rpm).

11.2.6 Add 1.1 mL of 1.25 M glycine. Mix once by inversion.

11.2.7 Centrifuge cells at 1000 rpm for 5 min. Discard supernatant.

11.2.8 Wash cells by resuspending them in 10 mL of ice-cold PBS in a 15 mL – 50 mL conical tube and centrifuge at 1000 rpm for 5 min. Carefully discard supernatant.

11.3 **Cell Lysis – for all cells**

11.3.1 Add Working Lysis Buffer (1X) to the cell pellet and resuspend by carefully pipetting up and down:

Adherent cells = 200 µL/ $1x10^6$ cells
Suspension cells = 100 µL/$1x10^6$ cells

11.3.2 Transfer cell suspension to a 1.5 mL vial and incubate on ice for 10 min.

11.3.3 Vortex vigorously for 10 sec and centrifuge at 5000 rpm for 5 min.

11.3.4 Carefully remove supernatant from centrifuged samples. Keep a sample of the supernatant for further analysis.

11.3.5 Add Working Extraction Buffer to chromatin pellet and resuspend by carefully pipetting up and down (50 µL/$1x10^6$ cells, 500 µL maximum for each vial).

11.3.6 Incubate the sample on ice for 10 min and vortex occasionally.

11.3.7 Resuspend the sample and sonicate 2 X 20 seconds to increase chromatin extraction. Allow the sample to cool on ice between sonication pulses for 30 seconds.

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

11.3.9 Transfer supernatant to a new vial.

11.3.10 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. To store chromatin, snap freeze by dropping aliquots in liquid nitrogen and quickly storing tubes at –80°C freezer. Avoid multiple freeze/thaw cycles.

11.4 **Protocol for Tissues**

11.4.1 Put the tissue sample into a 60 – 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample.

11.4.2 Weigh the sample and cut the sample into small pieces (1-2 mm^3) with a scalpel or scissors. Tissue should be cut in a petri dish resting on a block of dry ice to prevent sample degradation by proteases.

***Note**: For tissues that are not cross-linked, go directly to Step 11.5.1.*

11.4.3 **For cross-linked tissues only:** Transfer tissue pieces to a 15 mL – 50 mL conical tube.

11.4.4 Add 1 mL of cross-link solution for every 40 mg tissues and resuspend by pipetting up and down carefully.

11.4.5 Incubate at room temperature for 15-20 min on a rocking platform.

11.4.6 Add 1.1 mL of 1.25 M glycine for every 10 mL of crosslink solution.

11.4.7 Mix once by inversion and centrifuge at 1000 rpm for 5 min. Discard the supernatant.

11.4.8 Wash tissue pieces once by resuspending them in 10 mL of ice-cold PBS once by centrifugation at 1000 rpm for 5 min. Discard the supernatant.

11.5 **Cell Lysis and Chromatin Extraction**

11.5.1 Transfer tissue pieces to a Dounce homogenizer.

11.5.2 Add 1 mL Working Lysis Buffer for every 200 mg tissues (or 0.2 mL Working Lysis Buffer for every 40 mg tissues).

11.5.3 Disaggregate tissue pieces by 10 – 20 strokes.

11.5.4 Transfer homogenized mixture to a 15 mL -50 mL conical tube and centrifuge at 3000 rpm for 5 min 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.

11.5.5 Carefully remove supernatant from centrifuged samples. Keep a sample of the supernatant for further analysis.

11.5.6 Add Working Extraction Buffer to chromatin pellet and resuspend by carefully pipetting up and down (50 µL/ 40 mg tissue, 500 µL maximum for each vial).

11.5.7 Incubate the sample on ice for 10 min and vortex occasionally.

11.5.8 Resuspend the sample and sonicate 2 X 20 seconds to increase chromatin extraction. Allow the sample to cool on ice between sonication pulses for 30 seconds.

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

11.5.10 Transfer supernatant to a new vial.

11.5.11 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. To store chromatin, snap freeze by dropping aliquots in liquid nitrogen and quickly storing tubes at –80°C freezer. Avoid multiple freeze/thaw cycles.

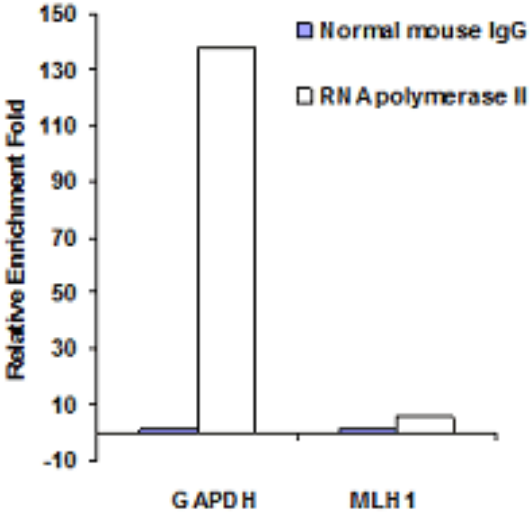


Figure 1. ChIP analysis of RNA polymerase II enriched in GAPDH and MLH1 promoters using ChIP Kit One-Step (ab117138). Chromatin extract was prepared from formaldehyde fixed colon cancer cells ($2x10^5$ cells) using ab117152.

12. TROUBLESHOOTING

Problem	Cause	Solution
Low yield of chromatin	Insufficient amount of samples.	To obtain the best results, use 1 x10^6 - 5x10^6 cells, or 50 - 200 mg tissues per ChIP reaction.
	Insufficient chromatin extraction.	Ensure all reagents have been added at 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.	Ensure that the kit has not exceeded expiration date, as expired reagents may cause inefficient extraction.
Low yield of chromatin	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 (keep for 3-6 months). Avoid multiple freeze/thaw cycles.

13. NOTES

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