

Version 3b Last updated 18 March 2026

# **ab223876**

# **Chromatin Extraction**

# **Kit – Flexible Format**

For the extraction of chromatin from cells and tissue.

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

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# 1. Overview

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. Protocol Summary

Prepare cell suspension / homogenize tissue



Formaldehyde cross-linking



Chromatin extraction



Analysis of chromatin fragmentation

### 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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

### 4. Storage and Stability

**Store kit as given in the table upon receipt. Equilibrate Formaldehyde Quenching Solution (10X) to RT before use.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

### 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

## 6. Materials Supplied

Item	Quantity	Storage temperature
Extraction and Lysis Buffer	100 mL	4°C
Formaldehyde Quenching Solution (10X)	50 mL	4°C
IP Buffer Concentrate (11X)	5 mL	4°C
Protease Inhibitor Cocktail	0.5 mL	-20°C
Stringency Supplement	5 mL	4°C

## 7. Materials Required, Not Supplied

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

- PBS
- 1% formaldehyde in PBS, may be prepared by diluting 37% stock in PBS shortly before cross-linking. **Note that formaldehyde is volatile and toxic, follow your organization's health and safety regulation when preparing, handling and disposing of this chemical.**
- Benchtop centrifuge
- Sonicator
- Thermocycler
- Equipment for DNA agarose electrophoresis

For DNA fragmentation analysis:

- Proteinase K
- Qiagen PCR Clean Up Kit
- RNase A 0.1 mg/mL
- 3M NaCl

## 8. Technical Hints

- **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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Reagent volumes in the protocols below are provided per extraction ( $1 \times 10^6$  cells or 40 mg of tissue). Scale the volumes up if performing a large-scale extraction.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

## 9. Preparation of starting material

### 9.1 From adherent cells:

Adherent cells can be cross-linked either directly on the plate or harvested by trypsinization first and then cross-linked from cell pellet. For direct cross-linking refer to section 10.2, for trypsin harvesting refer to section 9.1.1.

- 9.1.1 Grow cells to 70-80% confluency on a culture plate or flask (For example  $1 \times 10^7$  HeLa cells seeded in a triple flask would yield  $10 \times 10^7$  cells). Remove the growth medium. Wash the cells twice with room temperature PBS then remove PBS.
- 9.1.2 Add 1 mL of room temperature PBS per 20 cm<sup>2</sup> area of cells (e.g. add 3 mL of PBS to a 100 mm plate) and scrape cells into a 15 mL tube.  
Alternatively, dispense enough trypsin/EDTA solution to completely cover the monolayer of cells and incubate in a 37°C incubator for about 2 minutes or until cells detach from the surface. When the trypsinization process is complete, the cells will be in suspension and appear rounded. Add serum or media containing serum to the cell suspension as soon as possible to protect cells from damage caused by tryptic activity.  
**Δ Note:** trypsinization may have an impact on the cellular pathway of interest.
- 9.1.3 Count cells using a hemocytometer.
- 9.1.4 Centrifuge the cells for 5 minutes at 200 g and 4°C and discard the supernatant.
- 9.1.5 Wash cell pellet once with ice-cold PBS and resuspend the cells in 10 mL ice-cold PBS.
- 9.1.6 Transfer at least  $1 \times 10^6$  cells into a clean tube.
- 9.1.7 Centrifuge for 5 minutes at 200 g and 4°C. Higher speed may be required for some cell types.
- 9.1.8 Discard the supernatant.
- 9.1.9 Cell pellet is ready for cross-linking, proceed to Section 10 immediately.

### 9.2 From suspension cells:

- 9.2.1 Grow cells to at least  $2 \times 10^6$  cells/mL density and collect the cells in a 15-mL tube.
- 9.2.2 Centrifuge the cells for 5 minutes at 200 g and 4°C and discard the supernatant.

- 9.2.3 Wash cell pellet once with ice-cold PBS and resuspend cells in 10 mL of ice-cold PBS.
- 9.2.4 Transfer  $1 \times 10^6$  cells into a clean tube.
- 9.2.5 Centrifuge for 5 minutes at 200 g and 4°C. Higher speed may be required for some cell types.
- 9.2.6 Discard the supernatant.
- 9.2.7 Cell pellet is ready for cross-linking, proceed to Section 10 immediately.

### **9.3 From tissues:**

This protocol is suitable for both fresh and frozen tissues.

- 9.3.1 Weigh 40 mg of tissue and cut it into small pieces (approximately 1 mm<sup>3</sup>) on a cold petri dish to minimize sample degradations.
- 9.3.2 Wash the tissue twice with ice-cold PBS.
- 9.3.3 Discard the supernatant.
- 9.3.4 The tissue is ready for cross-linking, proceed to Section 10 immediately.

## 10. Formaldehyde cross-linking

Shortly before cross-linking prepare 1% formaldehyde in PBS, keep at room temperature. This may be achieved by diluting 37% formaldehyde stock in PBS. You will need 1 mL of 1% formaldehyde per  $2 \times 10^6$  cells or 20 mg of tissue. In case of direct cross-linking of adherent cells on a plate you will need 10 mL of 1% formaldehyde per 100 mm plate. **Note that formaldehyde is volatile and toxic, follow your organization's health and safety regulation when preparing, handling and disposing this chemical.**

Shortly before cross-linking prepare 1X Formaldehyde Quenching Solution in PBS by combining 1 part of Formaldehyde Quenching Concentrate (10x) and 9 parts of PBS, keep at room temperature. You will need 1 mL of Formaldehyde Quenching Solution per  $2 \times 10^6$  cells or 40 mg of tissue. In case of direct cross-linking of adherent cells on a plate you will need 10 mL of Formaldehyde Quenching Solution per 100 mm plate.

### 10.1 Cross-linking of harvested cells or diced tissue

- 10.1.1 Thoroughly resuspend cell pellet or diced tissue in room temperature 1% formaldehyde. Use 1 mL of 1% formaldehyde per  $2 \times 10^6$  cells or 40mg of tissue.
- 10.1.2 Incubate cells/tissue at room temperature for 12 min with mixing, preferably on a rotating wheel or a seesaw platform.
- 10.1.3 Centrifuge suspension for 3 minutes at 1000 g.
- 10.1.4 Remove as much formaldehyde solution from cells as possible.
- 10.1.5 Thoroughly resuspend cell pellet or diced tissue in room temperature 1X Formaldehyde Quenching Solution. Use 1 mL of Formaldehyde Quenching Solution per  $2 \times 10^6$  cells or 20 mg of tissue.
- 10.1.6 Incubate cells/tissues at room temperature for 5 min with mixing, preferably on a rotating wheel or a seesaw platform.
- 10.1.7 Centrifuge suspension for 3 minutes at 1000 g.
- 10.1.8 Remove as much Formaldehyde Quenching Solution from cells as possible.
- 10.1.9 Wash cross-linked cells or tissue twice by resuspending the pellet in PBS and centrifuging suspension for 3 minutes at 1000 g. Use 1 mL of PBS per  $2 \times 10^6$  cells or 40 mg of tissue.
- 10.1.10 Discard the supernatant after the final PBS wash.

10.1.11 Cross-linked cells/tissue pellet can be frozen at -80C or processed directly for chromatin extraction, (refer to section 11 for chromatin extraction). Freezing cells/tissue is recommended as it enables more convenient and flexible workflow.

## **10.2 Direct cross-linking of adherent cells without harvesting**

- 10.2.1 Grow cells to 70-80% confluency on a culture plate or flask (For example 1E7 HeLa cells seeded in a triple flask would yield 10E7 cells). Remove the growth medium. Wash the cells twice with room temperature PBS then remove PBS.
- 10.2.2 Add 3 mL of room temperature 1% formaldehyde per 20 cm<sup>2</sup> plate surface area, e.g. 9 mL for 100 mm plate.
- 10.2.3 Incubate plate at room temperature for 15 min with mixing, preferably on a seesaw platform.
- 10.2.4 Remove as much formaldehyde solution from cells as possible.
- 10.2.5 Add 3 mL of room temperature Formaldehyde Quenching Solution per 20 cm<sup>2</sup> plate surface area, e.g. 9 mL for 100 mm plate.
- 10.2.6 Incubate plate at room temperature for 5 min with mixing, preferably on a seesaw platform.
- 10.2.7 Remove as much Formaldehyde Quenching Solution from cells as possible.
- 10.2.8 Wash the cells twice with room temperature PBS then remove PBS.
- 10.2.9 Add 1 mL of room temperature PBS per 20 cm<sup>2</sup> plate surface area, e.g. add 3 mL of PBS to a 100 mm plate. Scrape cells into a 15 mL tube.
- 10.2.10 Count cells using a hemocytometer.
- 10.2.11 Transfer at least 1x10<sup>6</sup> cells into a clean tube.
- 10.2.12 Centrifuge the cells for 2 minutes at 500 g.
- 10.2.13 Discard the supernatant.
- 10.2.14 Cross-linked cells pellet can be frozen at -80C or processed directly for chromatin extraction, (refer to section 11 for chromatin extraction). Freezing cells/tissue is recommended as it enables more convenient and flexible workflow.

## 11. Chromatin Extraction

Suitable for both fresh and frozen cross-linked cells/tissues. To minimize variability between the experiments process either fresh or frozen material every time. Working with frozen cross-linked materials is recommended as it enables more convenient and flexible workflow.

Depending on the specific type of sonicator available in your laboratory it may be impractical to use buffer volumes indicated in the protocol(s) below. In this case scale up or down as appropriate keeping cells concentration constant.

When using the kit for the first time with a new cell line or tissue, optimization of sonication conditions will be required. Typically, sonication time and intensity are subject to optimization. For further advice refer to Pchelintsev NA, Adams PD, Nelson DM "Critical Parameters for Efficient Sonication and Improved Chromatin Immunoprecipitation of High Molecular Weight Proteins" 2016, PLoS ONE 11(1): e0148023. doi:10.1371/journal.pone.0148023.

**Δ Note:** Procedures are applicable to  $10 \times 10^6$  cross-linked cells or 20 mg cross-linked tissue.

### 11.1 Standard Procedure:

- 11.1.1 Add 5  $\mu$ L of 200x protease inhibitors cocktail to 1 mL of Extraction and Lysis Buffer. Mix well and keep at room temperature.
- 11.1.2 Resuspend  $10 \times 10^6$  cross-linked cells or 40 mg cross-linked tissue in 500  $\mu$ L of Extraction and Lysis Buffer supplemented with protease inhibitors. In case of cross-linked tissue, use Dounce homogenizer to accomplish sample homogenization.
- 11.1.3 Incubate sample from 11.1.2 at room temperature for 30 min with mixing, preferably on a rotating wheel or 1000 rpm vortex shaker.
- 11.1.4 Optional – at this point cell concentration can be measured again using a hemocytometer. This may be particularly useful for tissue samples or adherent cells cross-linked directly on a plate.
- 11.1.5 Centrifuge the suspension for 3 minutes at 1000 g.
- 11.1.6 Remove supernatant.

- 11.1.7 Resuspend pellet in 500  $\mu$ L of Extraction and Lysis Buffer supplemented with protease inhibitors.
- 11.1.8 Sonicate samples to shear chromatin to DNA fragments sized between 100 bp and 700 bp. Section 12 describes how to assess DNA fragmentation by agarose gel electrophoresis.  
**Δ Note:** With Diagenode water bath sonicators (Bioruptor series) 10-15 minutes of non-stop sonication at L-power typically produce desired results. Make sure to provide enough cooling to the sample so that its temperature does not exceed +25C. In case of water bath sonicators fill the bath with ice-cold water but avoid floating ice which may shield tubes with samples.
- 11.1.9 Following fragmentation analysis, if chromatin fragments are distributed between 100 bp and 700 bp, the ultrasound treatment has been sufficient and you may proceed to the next step. If high proportion of chromatin fragments are larger than 700 bp, additional sonication is required.
- 11.1.10 Add 10  $\mu$ L of IP Buffer Concentrate for every 100  $\mu$ L of sonicated chromatin. For example, if you used 20  $\mu$ L out of 500  $\mu$ L of sonicated chromatin for fragmentation analysis, you should have 480  $\mu$ L of chromatin left and therefore add 48  $\mu$ L IP Buffer Concentrate.
- 11.1.11 The resulting chromatin can be used directly for chromatin immunoprecipitation or frozen at -80C.

## **11.2 Extra stringent procedure for difficult to sonicate samples:**

- 11.2.1 Add 2.5  $\mu$ L of 200x protease inhibitors cocktail and 50  $\mu$ L of Stringency Supplement (11x) to 0.5 mL of Extraction and Lysis Buffer. Mix well and keep at room temperature. This is Stringent Extraction Buffer supplemented with protease inhibitors.
- 11.2.2 Add 2.5  $\mu$ L of 200x protease inhibitors cocktail to 0.5 mL of Extraction and Lysis Buffer. Mix well and keep at room temperature. This is Lysis Buffer supplemented with protease inhibitors.
- 11.2.3 Resuspend  $10 \times 10^6$  cross-linked cells or 40 mg cross-linked tissue in 500  $\mu$ L of Stringent Extraction Buffer supplemented with protease inhibitors. In case of cross-linked tissue, use Dounce homogenizer to accomplish sample homogenization.
- 11.2.4 Incubate tube at room temperature for 30 min with mixing, preferably on a rotating wheel or 1000 rpm vortex shaker.

- 11.2.5 Optional – at this point cell concentration can be measured again using a hemocytometer. This may be particularly useful for tissue samples or adherent cells cross-linked directly on a plate.
- 11.2.6 Centrifuge the suspension for 3 minutes at 1000 g.
- 11.2.7 Remove supernatant. You are likely to find that the pellet significantly increased in size compared to the starting material. Take a note of pellet volume (approximately)
- 11.2.8 Add 1000  $\mu\text{L}$  of deionized water for every 100  $\mu\text{L}$  of pellet and resuspend by vortexing.
- 11.2.9 Centrifuge the suspension for 3 minutes at 1000 g.
- 11.2.10 Remove supernatant.
- 11.2.11 Resuspend pellet in 500  $\mu\text{L}$  of Lysis Buffer supplemented with protease inhibitors (prepared in step 6.2.2).
- 11.2.12 Sonicate samples to shear chromatin to DNA fragments sized between 100 bp and 700 bp. Section 12 describes how to assess DNA fragmentation by agarose gel electrophoresis.  
**Δ Note:** With Diagenode water bath sonicators (Bioruptor series) 10-15 minutes of non-stop sonication at L-power typically produce desired results. Make sure to provide enough cooling to the sample so that its temperature does not exceed +25C. In case of water bath sonicators fill the bath with ice-cold water but avoid floating ice which may shield tubes with samples.
- 11.2.13 Following fragmentation analysis, if chromatin fragments are distributed between 100 bp and 700 bp, the ultrasound treatment has been sufficient and you may proceed to the next step. If high proportion of chromatin fragments are larger than 700 bp, additional sonication is required.
- 11.2.14 Add 10  $\mu\text{L}$  IP Buffer Concentrate for every 100  $\mu\text{L}$  of sonicated chromatin. For example, if you used 20  $\mu\text{L}$  out of 500  $\mu\text{L}$  of sonicated chromatin for fragmentation analysis, you should have 480  $\mu\text{L}$  of chromatin left and therefore add 48  $\mu\text{L}$  IP Buffer Concentrate.
- 11.2.15 The resulting chromatin can be used directly for chromatin immunoprecipitation or frozen at -80C.

## 12. Analysis of chromatin fragmentation

If you would like to use express method, for every new cell line or tissue you should validate that both express and standard methods produce similar results. Standard method is more reliable and, in case of inconsistencies, should be the preferred way to assess size distribution of DNA fragments from sonicated chromatin.

### 12.1 Standard Method:

To 20  $\mu$ L of each sample:

- 12.1.1 Add 2  $\mu$ L of 3M NaCl, mix well.
- 12.1.2 Add 2  $\mu$ L of 1 mg/mL Proteinase K (at least 30 U/mg, e.g. Sigma P2308), mix well.
- 12.1.3 Incubate 8 hours (or overnight) at +60C.
- 12.1.4 Purify DNA using Qiagen PCR Clean up kit. Elute with 30  $\mu$ L EB.
- 12.1.5 Add 3  $\mu$ L of 1 mg/mL RNase A
- 12.1.6 Incubate 15 min at room temperature.
- 12.1.7 Add appropriate amount of sample buffer compatible with agarose gel electrophoresis. Avoid sample buffers containing bromophenol blue or other dyes that cause "UV-shadow". We recommend using the following mixture as 6x sample buffer: 60% glycerol, 20 mM Tris-HCl, pH 8.0, 0.12% m/v Orange G.
- 12.1.8 Analyze 15  $\mu$ L of the resulting mixtures on 1.5% agarose gel.

### 12.2 Express Method:

To 20  $\mu$ L of each sample:

- 12.2.1 Add 2  $\mu$ L of 3M NaCl, mix well.
- 12.2.2 Add 2  $\mu$ L of 1 mg/mL Proteinase K (at least 30 U/mg, e.g. Sigma P2308), mix well.
- 12.2.3 Incubate 15 min at +60C.
- 12.2.4 Add 25  $\mu$ L of water, mix well.
- 12.2.5 Add appropriate amount of sample buffer compatible with agarose gel electrophoresis. Avoid sample buffers containing bromophenol blue or other dyes that cause "UV-shadow". We recommend using the following mixture as 6x sample buffer: 60% glycerol, 20 mM Tris-HCl, pH 8.0, 0.12% m/v Orange G.
- 12.2.6 Analyze 20  $\mu$ L of the resulting mixtures on 1.5% agarose gel.

## 13. Troubleshooting

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
Low yield of chromatin	Insufficient amount of samples	To obtain best results, use 5-10E6 cells or 40-200 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 $-80^{\circ}\text{C}$ (keep for 3-6 months). Avoid multiple freeze/thaw cycles

## 14. Notes

## Technical Support

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