

# **ab185901 – Chromatin Accessibility Assay Kit**

## Instructions for Use

For the gene-specific analysis of chromatin accessibility including nucleosome/transcription factor positioning from various biological samples via real time PCR

[View kit datasheet: www.abcam.com/ab185901](http://www.abcam.com/ab185901)

(use [www.abcam.cn/ab185901](http://www.abcam.cn/ab185901) for China, or [www.abcam.co.jp/ab185901](http://www.abcam.co.jp/ab185901) for Japan)

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

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## 1. BACKGROUND

The accessibility of regulatory elements in chromatin is critical for many aspects of gene regulation. Nucleosomes positioned over regulatory elements inhibit access of transcription factors to DNA. To elucidate the role of the interactions between chromatin and transcription factors, it is crucial to determine chromatin accessibility through mapping of the nucleosome positioning along the genome. In general, the more condensed the chromatin, the more difficult it is for transcription factors and other DNA binding proteins to access DNA and carry out their tasks. The more accessible the DNA, the more likely surrounding genes are actively transcribed. The presence (or the absence) of nucleosomes directly or indirectly affects a variety of other cellular and metabolic processes such as recombination, replication, centromere formation, and DNA repair.

There are several methods currently used for detecting chromatin accessibility. The traditional method is the use of DNase I or micrococcal nuclease as a probe for chromatin accessibility. However this method may have drawbacks to limit from broad-spectrum use. For example, this method requires a significant amount of starting material and introduces assay bias because of difficult control of enzyme concentrations and digestion time, as well as the inability to function in a harsh environment. In particular, the traditional method cannot be used for tissue samples. To address these problems Abcam offers the Chromatin Accessibility Assay Kit that allows for the analysis of chromatin accessibility in multiple gene promoters simultaneously. The kit has the following advantages and features:

The Chromatin Accessibility Assay Kit (ab185901) has the following advantages:

- Extremely fast and convenient protocol that allows the entire procedure (from cell tissue sample to ready-to-use DNA for PCR) to be finished in as short as 1 hour and 30 minutes.

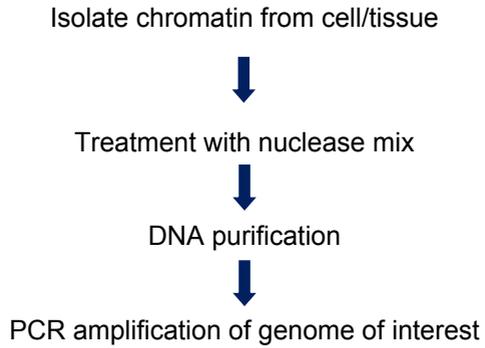
# INTRODUCTION

- Able to be used with cultured cells and also fresh and frozen tissues.
- Fast process minimizes nuclear damage and loss of disassociated chromatin components, preserving chromatin structure.
- Internal control primers are included in the kit as references for analyzing the extent of chromatin accessibility in a specified gene target in the sample DNA, and also for validating whether the proper enzymatic digestions are achieved.

The Chromatin Accessibility Assay Kit is a complete set of optimized reagents designed for conducting a gene-specific analysis of chromatin accessibility including nucleosome/transcription factor positioning from various biological samples via real time PCR.

The Chromatin Accessibility Assay Kit contains all the necessary reagents required for obtaining a gene-specific analysis of chromatin accessibility from cell/tissue samples via real time PCR. This kit includes internal control primers for determining whether chromatin digestion is successfully achieved. In this assay, chromatin is isolated from the cells/tissues and is treated with a nuclease mix. DNA is then isolated and amplified with real time PCR for region-specific analysis of chromatin accessibility. Chromatin states can be identified based on how accessible the DNA is to nucleases. The DNA in heterochromatin is inaccessible to outside proteins, including exogenous nucleases, rendering it protected by nucleosome or DNA/protein complexes and becomes available for subsequent PCR with insignificant Ct shifts between digested and undigested samples. In contrast, the DNA in euchromatin (nucleosome depletion) is accessible to exogenous nucleases, making it susceptible to nuclease digestion and becomes unavailable for PCR with a large Ct shift between digested and undigested samples.

## 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 away from light and as given in the table 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.

Check if Wash Buffer contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

**5. MATERIALS SUPPLIED**

Item	48 Tests	Storage Condition (Before Preparation)
10X Lysis Buffer	11 mL	RT
10X Wash buffer	10 mL	4°C
1000X Protease Inhibitor Cocktail	50 µL	4°C
Nuclear Digestion Buffer	3 mL	4°C
Nuclease mix	50 µL	-20°C
Reaction Stop Solution	0.5 mL	RT
Proteinase K, 10 mg/mL	110 µL	4°C
DNA Binding Solution	15 mL	RT
10X DNA Washing Solution	3 mL	RT
Elution Solution	1 mL	RT
Positive Control Primer-F (20 µM)	10 µL	-20°C
Positive Control Primer-R (20 µM)	10 µL	-20°C
Negative Control Primer-F (20 µM)	10 µL	-20°C
Negative Control Primer-R (20 µM)	10 µL	-20°C
F-Spin column	50	RT
F-Collection Tube	50	RT

### **6. MATERIALS REQUIRED, NOT SUPPLIED**

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

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Sonicator
- 1.5 mL microcentrifuge tubes
- Vortex mixer
- Dounce homogenizer with small clearance pestle
- Variable temperature waterbath or incubator oven
- Thermocycler with 48 or 96-well block
- Real Time PCR instrument
- Centrifuge (up to 14,000 rpm)
- 0.2 mL or 0.5 mL PCR vials
- 15 mL conical tube
- Cells or tissues
- Cell culture medium
- 100% Ethanol
- 1X PBS
- PCR Master Mix
- Distilled Water

## 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.**
- For 48 reactions, the maximal reactions for each control is 16 reactions (16 for negative, 16 for positive and 16 for target regions).

## 9. REAGENT PREPARATION

Prepare fresh reagents immediately prior to use.

### 9.1 **Working Lysis Buffer**

Add 10  $\mu$ L of Protease Inhibitor Cocktail to every 10 mL of Lysis Buffer required.

### 9.2 **1X Wash Buffer**

Add 5 mL of 10X Wash Buffer to 45mL of distilled water

### 9.3 **1X DNA Washing Solution**

Add 3 mL of 10X DNA Washing Buffer to 27 mL of 100% Ethanol.

## 10. SAMPLE PREPARATION

Input Amount of Cell/tissues: The amount of cells/tissues for each reaction can be from  $1 \times 10^5$  cells or 2 mg tissues to  $1 \times 10^6$  cells or 20 mg tissues. For an optimal reaction, the input chromatin amount should be about  $0.5 \times 10^6$  cells or 10 mg tissues.

### 10.1 **For Monolayer or Adherent Cells**

- 10.1.1 Grow cells (treated or untreated) to 80%-90% confluency on a 100 mm plate (about  $2 \times 10^6$  to  $4 \times 10^6$  cells;  $0.5 \times 10^6$  cells are required for each reaction), then trypsinize and collect them into a 15 mL conical tube. Count the cells using a hemocytometer if needed
- 10.1.2 Centrifuge the cells at 1000 rpm for 5 minutes. Discard the supernatant.
- 10.1.3 Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.

### 10.2 **For Suspension cells**

- 10.2.1 Collect cells (treated or untreated) into a 15 mL conical tube.  $1 \times 10^6$  cells are required for each reaction. Count cells using a hemocytometer if needed.
  - 10.2.2 Centrifuge the cells at 1000 rpm for 5 minutes. Discard the supernatant.
  - 10.2.3 Wash cells with 10 mL of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.
- 10.3 Tissue**
- 10.3.1 Put the tissue sample into a 60 or 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample.
  - 10.3.2 Weigh the sample and cut the sample into small pieces (1-2 mm<sup>3</sup>) with a scalpel or scissors.
  - 10.3.3 Transfer tissue pieces to a 15 mL conical tube (10 mg tissue is required for each reaction).
  - 10.3.4 Transfer tissue pieces to a Dounce homogenizer.
  - 10.3.5 Add 400  $\mu$ L of 1X Lysis Buffer for every 20 mg tissues.
  - 10.3.6 Disaggregate tissue pieces by 10-20 strokes.
  - 10.3.7 Transfer 200  $\mu$ L of homogenized mixture to a 1.5 mL vial as a sample and 200  $\mu$ L of homogenized mixture to another 1.5 mL vial as a no-Nuclease control. Centrifuge at 5000 rpm for 5 minutes at 4°C and then go directly to Step 11.1.5 of Assay Procedure below.

## **11. ASSAY PROCEDURE**

### **11.1 Cell Lysis and Chromatin Extraction**

- 11.1.1 Add 1X Lysis Buffer to re-suspend the cell pellet (400  $\mu\text{L}/1 \times 10^6$ ).
- 11.1.2 Transfer 200  $\mu\text{L}$  of cell suspension to a 1.5 mL vial as a sample and 200  $\mu\text{L}$  of cell suspension to another 1.5 mL vial as a no-Nuclease control.
- 11.1.3 Incubate the cell suspension on ice for 10 minutes.
- 11.1.4 Vortex vigorously for 10 seconds and centrifuge at 5000 rpm for 5 minutes.
- 11.1.5 Carefully remove supernatant and wash chromatin pellet one time with 1 mL of 1X Wash Buffer by resuspending the chromatin pellet and centrifuging at 3000 rpm for 5 minutes at 4°C (in bench top centrifuge).

### **11.2 Chromatin Digestion**

- 11.2.1 Wash the chromatin pellet one time with 0.5 mL of 1X Wash Buffer by resuspending the chromatin pellet and centrifuging at 3000 rpm for 5 minutes at 4°C (in bench top centrifuge).
- 11.2.2 Carefully remove supernatant.
- 11.2.3 Prepare the Nuclease reaction mixture as follows. For each reaction:

Tube	Sample	No-Nuclease Control
<b>Nuclear Digestion Buffer</b>	48 $\mu$ L	50 $\mu$ L
<b>Nuclease Mix</b>	2 $\mu$ L	0 $\mu$ L
<b>Total</b>	50 $\mu$ L	50 $\mu$ L

Mix and add 50  $\mu$ L of the mixture to the chromatin sample and No-Nuclease control vials accordingly.

- 11.2.4 Resuspend the chromatin pellet by pipetting 3-4 times.
- 11.2.5 Tightly cap the tube/wells and incubate at 37°C (water bath or thermal cycler) for 4 min.
- 11.2.6 After 4 minutes reaction, add 10  $\mu$ L of Reaction Stop Solution to each vial and incubate at 37°C for 10 min.
- 11.2.7 Add 2  $\mu$ L of Proteinase K to each vial and incubate at 60°C for 15 min.

### 11.3 DNA Clean-Up

- 11.3.1 Add 250  $\mu$ L of DNA Binding Solution to each column. Then transfer the samples from each PCR tube to each column containing the DNA Binding Solution. Cap the columns and centrifuge at 12000 rpm for 30 seconds. Remove columns from collection tubes and discard the flow through. Place columns back into collection tubes.
- 11.3.2 Add 200  $\mu$ L of 1X DNA Washing Solution to each column. Centrifuge at 12000 rpm for 30 seconds. Remove columns from collection tubes and discard the flow through.
- 11.3.3 Repeat step b of DNA Clean-Up two times.
- 11.3.4 Put spin column into a 1.5 mL microcentrifuge tube and centrifuge at 12000 rpm for 1 minute to dry the columns.

- 11.3.5 Insert each column into a new 1.5 mL microcentrifuge tube. Add 20  $\mu\text{L}$  of Elution Solution directly to each column's filter membrane. Centrifuge at 12000 rpm for 45 seconds to elute DNA.
- 11.3.6 DNA is now ready for use, or storage at or below  $-20^{\circ}\text{C}$  for up to 6 months.

## 11.4 qPCR Analysis of the Sample

To confirm if the samples are successfully digested and to analyze the user-specified gene targets, a real time qPCR can be performed with use of the internal control primers included in the kit. qPCR could be performed by using your own successful method. Newly designed primers should be verified for amplification efficiency by generating a standard curve with a series dilution of genomic DNA.

### 11.4.1 Prepare the PCR reactions.

Component	Amount ( $\mu\text{L}$ )	Final Concentration
PCR Master Mix (2X)	10	1 x
Positive Control Primer F	0.5	0.4-0.5 $\mu\text{M}$
Positive Control Primer R	0.5	0.4-0.5 $\mu\text{M}$
DNA Template	2	0.1ng – 0.1 $\mu\text{g}$
DNA/RNA-free $\text{H}_2\text{O}$	6 - 7 $\mu\text{L}$	
Total Volume	20 $\mu\text{L}$	

For the negative control, use DNA/RNase-free water instead of DNA template.

## 11.4.2 Program the Thermocycler

Cycle Step	Temperature	Time	Number of cycles
Activation	95°C	7 minutes	1
Cycling	95°C	10 seconds	40-45
	55°C	10 seconds	
	72°C	10 seconds	
Final Extension	72°C	60 seconds	1

## 12. ANALYSIS

### 12.1 Data analysis after qPCR

Fold enrichment (FE) can be calculated by simply using a ratio of amplification efficiency of the Nse-treated DNA sample over that of the No-Nse control sample.

$$FE = 2^{(Nse\ CT - noNse\ CT)} \times 100\%$$

Example calculation:

If Ct for NoNse is 30 and the Nse sample is 34, then:

$$FE\ of\ Nse\ -untreated\ sample = 2^{(34-30)} \times 100\% = 1600\%$$

The experiments are considered to be appropriate and successful if the following is achieved: With positive control primers, FE% of the untreated sample should be in general greater than 1600% compared to the Nse-treated samples, which indicates the samples are properly digested and the targeted gene region is in the opened chromatin. With Negative control primers, FE% of the untreated samples should not be greater than 400% compared to Nse-treated samples, which indicates the targeted gene region is in the closed chromatin that is not digested.

Similarly, with the primers for user-specified gene targets, FE% of the untreated samples >1600% compared to the Nuclease-treated samples which indicates the gene region is in the opened chromatin, while FE% of the untreated samples <400% compared to the Nuclease-treated samples represents the gene region in closed chromatin.

**13. TROUBLESHOOTING**

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Low yield of chromatin	Insufficient amount of samples	To obtain the best results, the amount of samples should be $0.5 \times 10^6$ to $1 \times 10^6$ cells, or 10 to 20 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 a microscope after the tissue/cell lysis step
		Ensure that the cell or tissue species are compatible with this extraction procedure
Incorrect temperature and/or insufficient incubation time during extraction	Ensure the incubation time and temperature described in the protocol are followed correctly	

## RESOURCES

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Chromatin is inappropriately digested	Excessive digestion of chromatin	Excessive digestion could be indicated by increased Ct shift of the negative control gene between the Nse-treated and untreated sample. Check if excess Nuclease or prolonged incubation time was used. If so, reduce the Nse amount and the incubation time
	Too little digestion of chromatin	Too little digestion could be indicated by reduced Ct shift of the positive control gene between the Nse-treated and untreated sample. Check if the Nse amount or incubation duration is insufficient. If so, increase the Nse amount and the incubation duration
Elute Contains Little or No DNA	Poor input DNA quality (degraded)	Check if DNA is degraded by running a gel
	Concentration of ethanol solution used for DNA clean-up is not correct	Use 90% ethanol for DNA clean-up
	Sample is not completely passed through the filter membrane of column.	Centrifuge for 1 minute at 12000 rpm or until the entire sample has passed through the filter membrane

## RESOURCES

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Poor Results in Downstream qPCR	Little or no PCR product even in the positive control	Ensure that all PCR components were added and that a suitable PCR program is used (PCR cycle should be >35)
		PCR primers and probes were not appropriate or were incorrectly designed. Ensure the primer and probes are suitable for amplification of the DNA region of interest and the target regions to be amplified are less than 250 bp
		Ensure the amount of template DNA used in PCR was sufficient
	Significant non-specific PCR products	Failed chromatin digestion. Ensure that all steps of the digestion and cleanup protocol were followed and that input DNA amount is within the recommended range
		Primers and probes are not specific for target genes. Check the primer and probe design

14. NOTES

# RESOURCES



# RESOURCES

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