Cat. # 5333

For Research Use

TaKaRa

EpiScope® Nucleosome Preparation Kit

Product Manual

v201904Da

Table of Contents

I. Description

Nucleosomes are the constituent units of chromatin in eukaryotic nuclei, consisting of genomic DNA wrapped around a core histone complex. Nucleosomes are not spaced at regular intervals on genomic DNA. Their positions are known to be closely related to chromatin structure and transcriptional regulation. In epigenetics research, which is an important area in the study of the regulation of gene expression, nucleosome positioning has become a subject of intense interest. In genes activated for transcription, the promoter region is free of nucleosomes, allowing the binding of transcriptional initiation factors. However, DNA methylation in the promoter region leads to formation of nucleosomes, preventing the binding of transcription factors and allowing transcriptional suppression.

This kit is designed for the preparation of nucleosomes from cultured mammalian cells. DNA can be extracted from the prepared nucleosome and analyzed using a real-time PCR assay or high-speed sequencing to map nucleosome positions.

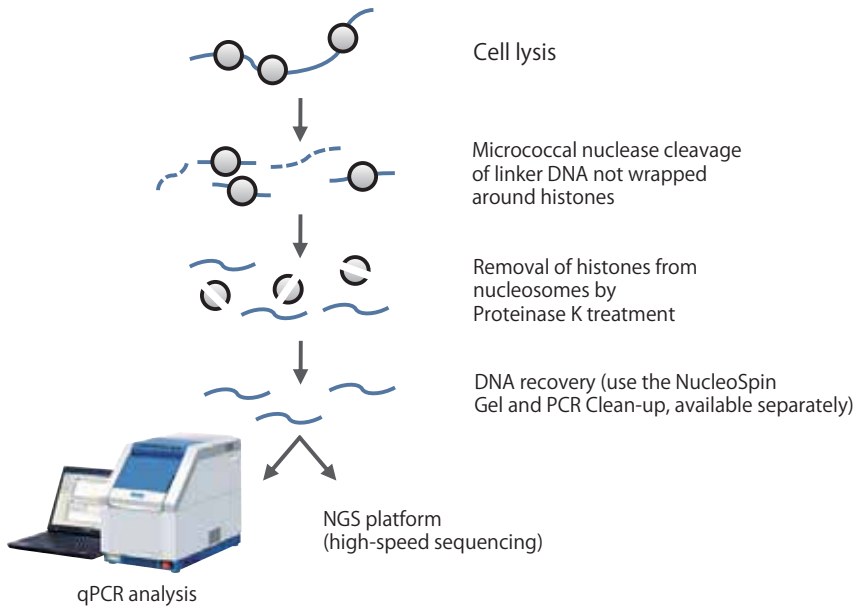
Protocol Overview:

1. Cell lysis under gentle conditions and collection of nuclei by centrifugation
2. Micrococcal nuclease cleavage of linker DNA (not wrapped around histones)
3. Removal of histones from nucleosomes by treatment with Proteinase K
4. DNA recovery using a DNA purification kit such as the NucleoSpin Gel and PCR Clean-up (sold separately; Cat. #740609.10/.50/.250)

Recovered DNA corresponds to genomic DNA regions that were incorporated into nucleosomes. The genomic DNA regions not wrapped around histones are cleaved by micrococcal nuclease.

Note:

This kit is not designed to fragment chromatin in cells exposed to cross-linking agents.



II. Components [for 50 reactions]

Package 1:

3.	Micrococcal Nuclease (20 U/μl)	50 μl
4.	Micrococcal Nuclease Buffer (10X)	1 ml
5.	RNase A (20 mg/ml)	100 μl
6.	0.5 M EDTA	100 μl
7.	Proteinase K	200 μl
8.	LINE-1 qPCR Primer (10 μM each)*	50 μl

Package 2:

1.	Cytoplasmic Lysis Buffer	25 ml x 2
2.	Protease Inhibitor Cocktail (100X)	550 μl

* This component is to be used as a reference for making corrections to the amounts of genomic DNA in relative quantitation analyses by real-time PCR. It is a primer for human genomic DNA detection and cannot be used for other species.

III. Storage

Package 1: –20℃
Package 2: 4℃

IV. Materials Required but not Provided

- Thermal cycler
  e.g., TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)*
- DNA purification kit
  e.g., NucleoSpin Gel and PCR Clean-up (Cat. # 740609.10/.50/.250)
- Electrophoresis apparatus
  e.g., Mupid-exU (Cat. #EXU-1)

* Not available in all geographic locations. Check for availability in your area.

V. Precautions for Use

This section describes precautions for using this kit. Read before use.

1. Gently spin down enzymes before use. Keep enzymes on ice while performing the protocol.
2. Thaw the Protease Inhibitor Cocktail, Micrococcal Nuclease Buffer (10X), and 0.5 M EDTA at room temperature. Spin down gently before use. Handle these reagents at room temperature while performing the protocol, and return to the appropriate storage temperatures after use.
3. Prepare the micrococcal nuclease reaction master mix at room temperature. Add the Micrococcal Nuclease last. (Salt will precipitate if the mixture is prepared on ice. Should precipitate form, incubate at 37℃ briefly to dissolve.)

VI. Protocol

A. Cell harvest and lysis

1. Harvest cells from culture plates and suspend in an appropriate amount of PBS to a final concentration of approximately 1 x 10^6 cells/ml. Dispense 1-ml aliquots of the suspension into 1.5-ml microtubes.

   ＊ Use approximately 0.5 to 2 x 10^6 cells per reaction.

2. Centrifuge at 3000 rpm, 4℃ for 3 min to collect the cells.
3. Remove the supernatant. Add 10 µl of the Protease Inhibitor Cocktail.
4. Add 1 ml of the Cytoplasmic Lysis Buffer. Resuspend well by pipetting.
5. Incubate on ice for 10 min.
6. Centrifuge at 5200 rpm, 4℃ for 10 min. While the cell lysate is being centrifuged, dilute the 10X Micrococcal Nuclease Buffer with sterile purified water to make 100 µl of a 1X Micrococcal Nuclease Buffer solution.
7. Remove the supernatant after centrifugation.
8. Add 50 µl of 1X Micrococcal Nuclease Buffer. Resuspend the nuclei-containing pellet by pipetting to yield a cell nuclei suspension.

   ＊ Thoroughly resuspend the nuclei-containing pellet by pipetting. The presence of a few residual pellet clumps will not affect the subsequent reaction.

B. Micrococcal nuclease treatment

1. At room temperature, prepare enough master mix for the number of reactions being performed plus some additional volume to account for pipetting error.

   <Per reaction>

Reagent	Volume
Micrococcal Nuclease (20 U/µl)	0.2 µl
Micrococcal Nuclease Buffer (10X)	5.0 µl
RNaseA (20 mg/ml)	2.0 µl
Protease Inhibitor Cocktail (100X)	1.0 µl
Sterile purified water	41.8 µl
Total	50 µl

Note:

- Salt will precipitate when the master mix is prepared on ice. Prepare the master mix at room temperature.
- Add the Micrococcal Nuclease immediately before starting the reaction.

2. Dispense 50 µl of the master mix into a 0.2-ml microtube, and then add 50 µl of the cell nuclei suspension from Step 8 of the section above entitled "A. Cell harvest and lysis".
3. Incubate at 37℃ for 30 min using a thermal cycler.
4. Add 2 µl of the 0.5 M EDTA to the microtube to stop the reaction.
5. Add 4 µl of the Proteinase K. Incubate at 37℃ for 30 min using a thermal cycler. Continue with the DNA extraction protocol in the section below entitled "C. DNA extraction".

C. DNA extraction

1. Using the NucleoSpin Gel and PCR Clean-up (sold separately), extract DNA from the Proteinase K-treated sample prepared in Step 5 of the section above entitled "B. Micrococcal nuclease treatment".
   (1) Add 212 μl of the Buffer NTI to 106 μl of the Proteinase K-treated sample.
   (2) Set the NucleoSpin Gel and PCR Clean-up Column in a collection tube (2 ml). Add the solution from (1) to the column and centrifuge at 11,000*g* for 1 min. Discard the filtrate and set a column in the same collection tube.
   (3) Add 700 μl of the Wash Buffer NT3 (containing ethanol) to the column and centrifuge at 11,000*g* for 1 min. Discard the filtrate and set a column in the same collection tube.
   (4) Centrifuge the column at 11,000*g* for 2 min.
   (5) Set a column in a 1.5 ml microtube (not supplied). Add 50 μl of the Buffer NE and incubate at room temperature for 1 min, followed by centrifugation at 11,000*g* for 1 min.
2. Analyze an aliquot (5 μl) of the DNA solution by agarose gel electrophoresis to verify the DNA size distribution. (Refer below to VII. Appendix, Subsection 2.)

VII. Appendix

1. Nucleosomal DNA analysis by real-time PCR

When assaying nucleosomal DNA prepared with this kit by real-time PCR, the DNA was extracted with approximately 50 μl of eluate (Buffer NE) of the NucleoSpin Gel and PCR Clean-up as described in Section "C. DNA extraction". In general, use 2 μl of the DNA eluate as template for real-time PCR. The real-time PCR assays performed with TB Green® *Premix Ex Taq*™ GC (Perfect Real Time) and the Thermal Cycler Dice Real Time System *II* are described below.

1. Prepare the following reaction mixture on ice.

Regent	Volume
TB Green *Premix Ex Taq* GC (2X)	12.5 μl
Forward Primer (10 μM)	0.5 μl
Reverse Primer (10 μM)	0.5 μl
Template	2.0 μl
Sterile purified water	9.5 μl
Total	25 μl

2. Start the reaction.

Start the reaction according to the following cycling conditions. Optimize the PCR conditions if necessary.
95℃ for 30 sec → (95℃ for 5 sec/60℃ for 30 sec) x 40 cycles → melting curve analysis

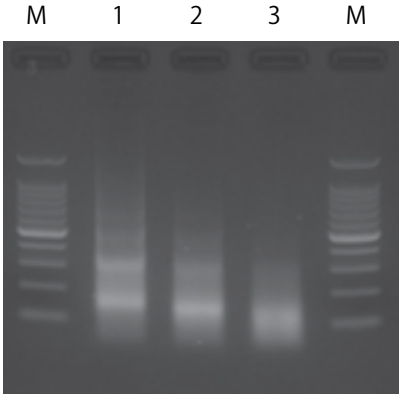
2. Amount of micrococcal nuclease and DNA size distribution

[Method]

HeLa cells (1 x 10^6 cells) were subjected to treatment according to the protocol for this kit. Various amounts of Micrococcal Nuclease were used (0.02 μl, 0.05 μl, and 0.2 μl). DNA was extracted with the NucleoSpin Gel and PCR Clean-up, yielding 50 μl of DNA solution, of which 5 μl was analyzed by agarose gel electrophoresis.

[Results]

For samples treated with lower amounts of Micrococcal Nuclease (0.02 μl, 0.05 μl), the electrophoretic results showed bands for mononucleosomal, dinuclepsomal, and trinucleosomal DNA fragments. For samples treated with 0.2 μl of Micrococcal Nuclease, mostly mononucleosomes were evident. In addition, the higher the amount of Micrococcal Nuclease used, the shorter the size of the mononucleosome-derived DNA fragment observed. This result may reflect the trimming activity at both ends of the DNA (Clark DJ. *J Biomol Struct Dyn.* (2010) **27:** 781-793).



1. Micrococcal Nuclease 0.02 μl
2. Micrococcal Nuclease 0.05 μl
3. Micrococcal Nuclease 0.2 μl
M. 100 bp DNA Ladder

2% Agarose L03
* Mononucleosomal DNA

Figure 2. Analysis of Nucleosomal DNA Size Distribution

VIII. Troubleshooting

If inadequate cleavage is observed, the following actions may improve results.

1. Use proper amounts of Micrococcal Nuclease. Using lower amounts of Micrococcal Nuclease will result in inadequate cleavage of DNA (Appendix-2).
2. Thoroughly resuspend the nuclei-containing pellet in 1X Micrococcal Nuclease Buffer during Step A-8, Section VI. Insufficient resuspension may affect the efficiency of DNA cleavage by Micrococcal Nuclease.

IX. Related Products

NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250)
TB Green® *Premix Ex Taq*™ GC (Perfect Real Time) (Cat. #RR071A/B)＊1, 2
Micrococcal Nuclease (Cat. #2910A)

＊1 We have begun the process of changing the names for Takara Bio's intercalator-based real-time PCR (qPCR) products to the "TB Green series". These products can be used the same way as before, as only the names are changing. Catalog number and product performance are unaffected by this transition.
＊2 Not available in all geographic locations. Check for availability in your area.

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