

Store at
-20°C

Micrococcal Nuclease

www.cellsignal.com

#10011

60 µl

Support: 877-678-TECH (8324)
www.cellsignal.com/supportOrders: 877-616-CELL (2355)
orders@cellsignal.comEntrez-Gene ID #3238436
UniProt ID #Q5HHM4

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: Micrococcal nuclease is a relatively non-specific endo-exonuclease derived from *Staphylococcus aureus*. Active in the pH range of 7.0-10.0, this product digests double-stranded, single-stranded, circular, and linear nucleic acids.

In the SimpleChIP® Enzymatic Chromatin IP kit assay, following cell lysis, the chromatin is fragmented by partial digestion with micrococcal nuclease to obtain chromatin fragments of 1 to 5 nucleosomes in size. Enzymatic digestion of chromatin is much milder than sonication and eliminates problems due to variability in sonication power and emulsification of chromatin during sonication, which can result in incomplete fragmentation of chromatin or loss of antibody epitopes due to protein denaturation and degradation.

This product is offered to conveniently provide additional micrococcal nuclease when fragmenting chromatin with our SimpleChIP® (#9002, #9003) and SimpleChIP® Plus (#9004, #9005) Enzymatic Chromatin IP Kits. These kits provide all the reagents required for performing 6 chromatin preparations (or optimizations) and 30 chromatin immunoprecipitation (ChIP) assays, however there are instances where extra micrococcal nuclease is desired.

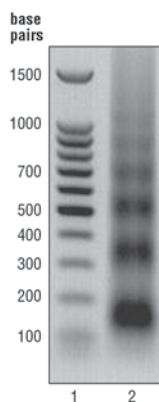
Method Overview

Cells are fixed with formaldehyde to cross-link histone and non-histone proteins to DNA.

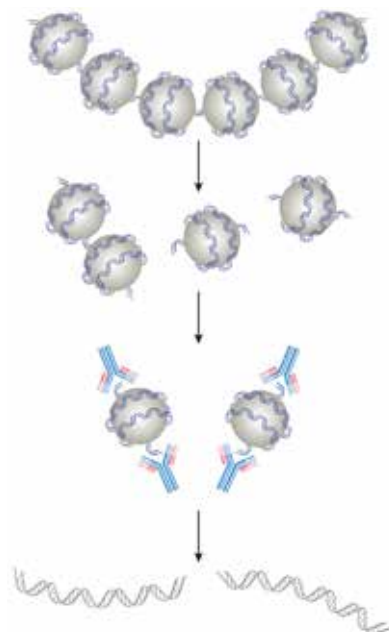
Chromatin is digested with Micrococcal Nuclease into 150-900 bp DNA/protein fragments.

Antibodies specific to histone or non-histone proteins are added and the complex co-precipitates and is captured by Protein G Agarose or Protein G magnetic beads.

Cross-links are reversed, and DNA is purified and ready for analysis.



HeLa cells were formaldehyde-crosslinked and chromatin was prepared and digested as described in Section A of the SimpleChIP® Kit protocol using Micrococcal Nuclease for digestion. DNA was purified as described in Section B. 10 µl were separated by electrophoresis on a 1% agarose gel (lane 2) and stained with ethidium bromide. Lane 2 shows that the majority of chromatin was digested to 1 to 5 nucleosomes (150 to 900 bp).



Storage: Supplied in 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA and 50% glycerol. Store at -20°C. Do not aliquot the product. This product is stable for 12 months.

Directions for Use: Use during chromatin fragmentation in chromatin immunoprecipitation (ChIP) assays as directed in the protocols for the SimpleChIP® and SimpleChIP® Plus Enzymatic Chromatin IP Kits. For proper chromatin digestion, micrococcal nuclease requires the use of SimpleChIP® Lysis Buffers A & B #14282.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Thank you for your recent purchase. If you would like to provide a review visit www.cellsignal.com/comments.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Optimization of Chromatin Digestion

Optimal conditions for digestion of cross-linked DNA to 150-900 base pairs in length depend on cell type and cell concentration as well as the concentration of Micrococcal Nuclease. Below is a protocol to determine the optimal digestion conditions for a specific cell type and concentration of cells (for more details, see protocol for SimpleChIP® #9002, 9003 & SimpleChIP® Plus (#9004, #9005) kits.)

1. Prepare cross-linked nuclei from 4×10^7 cells as described in Section A of the SimpleChIP® protocol, Steps 1-6.
2. Transfer 200 μ l of the nuclei preparation from Step 6 in Section A into 5 individual microcentrifuge tubes and place on ice.
3. Add 5 μ l Micrococcal Nuclease stock to 20 μ l of 1X Buffer B + DTT (1:5 dilution of enzyme).
4. To each of the 5 tubes in Step 2, add 0 μ l, 2.5 μ l, 5 μ l, 7.5 μ l or 10 μ l of the diluted Micrococcal Nuclease, mix by inverting tube several times and incubate for 20 min at 37°C with frequent mixing.
5. Stop digest by adding 20 μ l of 0.5 M EDTA and placing tubes on ice.
6. Pellet nuclei by centrifugation at 13,000 rpm in a microcentrifuge for 1 min at 4°C and remove supernatant.
7. Resuspend nuclear pellet in 200 μ l of 1X ChIP buffer + Protease Inhibitor Cocktail +PMSF. Incubate on ice for 10 min.
8. Sonicate lysate with several pulses to break nuclear membrane. Incubate samples 30 sec on wet ice between pulses.
 - Optimal conditions required for complete lysis of nuclei can be determined by observing nuclei under light microscope before and after sonication.
 - HeLa nuclei were completely lysed after 3 sets of 20-second pulses using a VirTis Virsonic 100 Ultrasonic Homogenizer/Sonicator set at setting 6 with a 1/8-inch probe.
 - Alternatively, nuclei can be lysed by homogenizing the lysate 20 times in a Dounce homogenizer; however, lysis may not be as complete.
9. Clarify lysates by centrifugation at 10,000 rpm in a microcentrifuge for 10 min at 4°C.
10. Transfer 50 μ l of each of the sonicated lysates to new microfuge tubes.
11. To each 50 μ l sample, add 100 μ l nuclease-free water, 6 μ l 5 M NaCl and 2 μ l RNase A. Vortex to mix and incubate samples at 37°C for 30 min.
12. To each RNase A-digested sample, add 2 μ l Proteinase K. Vortex to mix and incubate sample at 65°C for 2 hours.
13. Remove 20 μ l of each sample and determine DNA fragment size by electrophoresis on a 1% agarose gel with a 1 kb DNA marker.
14. Observe which of the digestion conditions produces DNA in the desired range of 150-900 base pairs (1 to 5 nucleosomes, see Figure 1). The volume of diluted Micrococcal Nuclease that produces the desired size of DNA fragments using this optimization protocol is equivalent to the volume of Micrococcal Nuclease stock that will need to be added to 4×10^7 cells to produce the desired size of DNA fragments. For example, if 5 μ l of diluted Micrococcal Nuclease produces DNA fragments of 150-900 base pairs in this protocol, then 5 μ l of stock Micrococcal Nuclease should be added to 4×10^7 cells during the digestion of chromatin in Step 7 of Section A.
15. If results indicate that DNA is not in the desired size range, then repeat optimization protocol, adjusting the amount of Micrococcal Nuclease in each digest accordingly.