

Product Information

Nuclease micrococcal from *Staphylococcus aureus*

With reaction buffer and BSA solution

CS0004

Product Description

CAS Number: 9013-53-0

Enzyme Commission (EC) Number: 3.1.31.1

Synonyms: Endonuclease micrococcal, MNase, Micrococcal endonuclease

Micrococcal Nuclease (MNase) is an endo-exonuclease that preferentially digests single-stranded nucleic acids. MNase was historically isolated from *Micrococcus pyogenes* var. *aureus* (now known as *Staphylococcus aureus*).^{1,2} The end products from MNase activity are mononucleotides and oligonucleotides with terminal 3'-phosphates. MNase is also active against double-stranded DNA and RNA, and all nucleic acids will be ultimately cleaved. MNase can be used for the limited digestion of chromatin to identify the location of nucleosomes.³

Applications of MNase include:

- Chromatin Immuno-Precipitation (ChIP) analysis⁴
- Nucleosome preparation
- Removal of nucleic acids present in protein preparation
- Reduction of viscosity of cell lysates during non-mechanical cell lysis preparation
- RNA sequencing and transcriptomic profiling

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

- Nuclease Micrococcal (Component CS0004A): 50 units, in solution [300-500 units/mL in 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, and 50% Glycerol]
- MNase Reaction Buffer, 10× (Component CS0004B): 1 mL [solution of 500 mM Tris (pH 8.0), with 50 mM CaCl₂]
- BSA (Component CS0004C): 1 mL (solution at 1 mg/mL BSA in water)

Storage/Stability

Store the kit components at -20 °C.

Preparation Instructions

For optimal activity, it is recommended to perform the reaction in 1× MNase reaction buffer with 0.1 mg/mL BSA. If dilution of enzyme is required:

1. Prepare fresh Diluent Solution (1× MNase reaction buffer with 0.1 mg/mL BSA solution). Store this Diluent Solution on ice.
2. Right before use, dilute the enzyme with the ice-cold Diluent Solution.

Procedure

- Nucleosome preparation can be performed as part of a ChIP procedure.
- Nucleosome fragmentation can be facilitated by a combination of sonication and digestion with MNase.

A generic procedure for nucleosome preparation is presented here. For specific materials and methodology, various publications in the literature may be consulted.⁵⁻⁸

Cell culture preparation and fixation

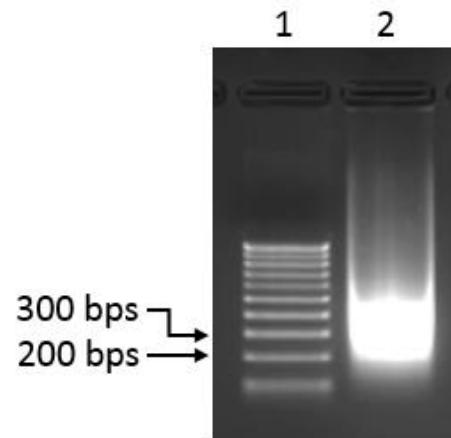
It is recommended to use this procedure with 4×10^6 to 5×10^6 cells. This assay was tested with HeLa cells and might require a calibration when other cells are used.

1. Crosslink cells:
 - 1.1. Add 1% formaldehyde for 10 minutes.
 - 1.2. Add 125 mM glycine for 2 minutes.
2. Remove the cell culture medium.
 - 2.1. Collect the cells.
 - 2.2. Centrifuge cells at $2000 \times g$ at 4°C for 5 minutes.
 - 2.3. Discard the supernatant.
 - 2.4. Continue to the next stage.

Nucleosome preparation

- Perform the MNase digest in a buffer composed of $1\times$ MNase Reaction Buffer (Component CS0004B) with 0.1 mg/mL BSA (Component CS0004C).
 - If another buffer is used, make sure that it contains 1-5 mM CaCl_2 .
1. Wash the cells according to the ChIP protocol of choice. Transfer the cells to CaCl_2 -containing buffer.
 2. Add 0.5 μL of Nuclease Micrococcal (MNase, Component CS0004A). Mix well. Incubate for 20 minutes at 37°C .
 3. Add 10 μL of 0.5 M EDTA to stop MNase activity. Incubate on ice for 2 minutes.
 4. Centrifuge at $12,000 \times g$ for 1 minute at 4°C . Discard the supernatant.
 5. Resuspend the pellet in 100-500 μL of buffer as required in microcentrifuge tube.
 6. Sonicate the resuspended pellet with a probe sonicator, to break the nuclear membrane. This stage must be calibrated according to specific assay conditions. As a starting point, perform three sonication pulses, 10 seconds each, with 10-second intervals on ice. This was found to produce a homogenous preparation of monomeric nucleosomes (see Figure 1).

Figure 1. Chromatin preparation analysis



HeLa cells were formaldehyde-crosslinked.

Chromatin was prepared and digested as described:

- A 50 μL sample of DNA was incubated with RNase and Proteinase K.
- The sample was purified using a microspin column (such as Catalogue Number NA1020), according to the column instructions.
- A sample of 10 μL was separated by electrophoresis on a 2% agarose gel (Lane 2) and stained with ethidium bromide. Lane 2 shows that the chromatin was digested to 1 to 2 nucleosomes in length.

References

1. Cunningham, L. *et al.*, *J. Am. Chem. Soc.*, **78(18)**, 4642-4645 (1956).
2. Telford, D.J., and Stewart, B.W., *Int. J. Biochem.*, **21(2)**, 127-138 (1989).
3. Chung, H.-R. *et al.*, *PLoS ONE*, **5(12)**, e15754 (2010).
4. Long, L. *et al.*, *J. Immunother. Cancer*, **9(12)**, e003973 (2021).
5. O'Neill, L.P., and Turner, B.M., *Methods Enzymol.*, **274**, 189-197 (1996).
6. Zeng, P.-Y. *et al.*, *BioTechniques*, **41(6)**, 694-698 (2006).
7. Roca, H., and Franceschi, R.T., *Nucleic Acids Res.*, **36(5)**, 1723-1730 (2008).
8. Cosseau, C., and Grunau, C., *Methods Mol. Biol.*, **791**, 195-212 (2011).

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