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) analysis[4]
- 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 $CaCl_2$]
- 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.

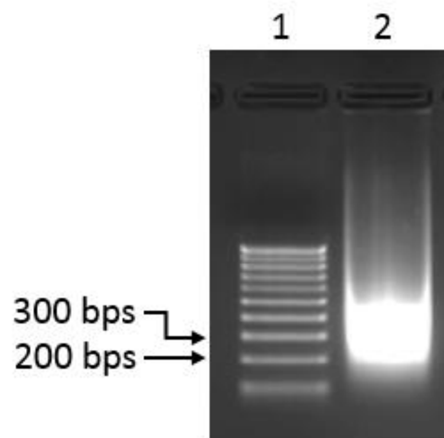
1. Crosslink cells:
    1.1. Add 1% formaldehyde for 10 minutes.
    1.2. Add 125 mM glycine for 2 minutes.
2. Remove the cell culture medium.
    2.1. Collect the cells.
    2.2. Centrifuge cells at 2000 × g at 4 °C for 5 minutes.
    2.3. Discard the supernatant.
    2.4. Continue to the next stage.

Nucleosome preparation

- Perform the MNase digest in a buffer composed of 1× 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 × 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

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