DNaseMe

(dsDNase)

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DNaseMe is a 42.8 kDa recombinant endonuclease, derived from marine amphipods, expressed in *Pichia pastoris*. The enzyme displays high specific activity towards double-stranded DNA leaving single-stranded DNA or RNA undamaged in standard conditions. DNaseMe is highly active in a broad spectrum of temperatures, buffer conditions and pH. The specific activity is similar to bovine DNase I however, DNaseMe is characterized by higher stability in demanding reaction and storage conditions (e.g. high salt and detergent containing buffers, elevated temperature). These features make DNaseMe extremely useful for rapid and "RNA safe" degradation of genomic DNA, where absence of ribonucleases is critical to maintain the integrity of RNA.

The enzyme hydrolyzes phosphodiester linkages yielding oligonucleotides with a 5'-phosphate and a 3'-hydroxyl groups.

Features and advantages

- → Active in a broad temperature range (10–80°C).
- → Active in a broad pH range (optimum at pH 6.0–9.0).
- → Highly active at elevated salt concentrations and other typical buffer additives (Table 1), which significantly improves efficiency and yield of various workflows.
- → Requires bivalent cations (Mg^{2+} and Ca^{2+}) for maximal activity.
- → Degrades dsDNA to fragments below 10 nt.
- → The activity towards dsDNA is at least 1000 times higher than towards ssDNA.

Variable / Parameter	Activity Range
pH	4.0–9.5 (optimum 6.0–9.0)
Temperature	10–80°C
Inactivation	85°C/15 min/1 mM DTT
Mg^{2+}	1–150 mM (Ca^{2+} increases the activity)
Ca^{2+}	1–50 mM
Ammonium sulfate	0–0.5 M
NaCl / KCl	0–2 M
Imidazole	0–0.4 M
Urea	0–4 M
Glycerol	0–50%
Triton X-100	0–20%
SDS	0–0.5% (not recommended)
DTT (at low temp.)	0–100 mM
β-mercaptoethanol	0–2.5%

Table 1. Operating conditions of DNaseMe

The enzyme retains its activity in a wide range of operating conditions and is active in the presence of high salt concentrations, detergents, reducing agents, imidazole and urea.

Applications

- → Extraction and purification of RNA (equivalent of DNase I).
- → Removal of contaminating genomic DNA from RNA samples.
- → Degradation of DNA template in transcription reactions.
- → Reduction of viscosity in biological samples.
- → Removal of residual DNA during primary stem cell isolation, biopharma and bioprocessing procedures.

Additional information

- The optimal concentration of DNaseMe in the final reaction mixture depends on several factors (level of nucleic acids contamination, temperature and time of incubation, salt concentration and other compounds present in the reaction mixture). The amount of DNaseMe and incubation conditions have to be determined experimentally (we recommend using 35 U DNaseMe for digestion of up to 50 µg dsDNA at 37°C for 10–15 min).
- In the presence of Mg^{2+} or Ca^{2+} ions, DNaseMe shows high endonuclease activity towards dsDNA leaving ssDNA and RNA essentially intact.
- Presence of Ca^{2+} ions increases the total activity of DNaseMe.
- In the presence of Mn^{2+} ions, the enzyme shows slightly elevated DNaseMe activity including activity towards ssDNA and RNA. Avoid using Mn^{2+} ions during purification of RNA or ssDNA.
- Inactivation of DNaseMe depends on the concentration of the reducing agent, inactivation time and temperature. We recommend inactivating DNaseMe by incubation at 85°C for 15 min in the presence of reducing agents such as DTT or TCEP (1–10 mM).
- **The enzyme requires 1–10 mM DTT or TCEP to be completely inactivated.**
- Alternatively, DNaseMe can be inactivated and removed by using a spin column or phenol/chloroform extraction.

Quality control

- → The purity >95% determined by densitometry of SDS-PAGE.
- → Undetectable RNase activity after incubation of 10 U DNaseMe with 1 µg of RNA for 1 hour at 37°C.
- → Undetectable proteolytic activity.

Unit Definition

One unit is defined as an increase in absorbance at 260 nm of 1.0 in 30 minutes at 37°C and pH 8.0 with herring sperm DNA as a substrate.

Storage buffer

20 mM Tris-HCl, pH 8.0; 50 mM KCl; 5 mM $MgCl_2$; 50% (v/v) glycerol

DNaseMe (dsDNase)

Component	EN33-250 25 000 U	EN33-050 5 000 U	EN33-S 500 U
DNaseMe (20 U/µl)	1250 µl	250 µl	25 µl

Additional information

Storage conditions

Store at -20°C in a freezer without a defrost cycle.
For long-term storage place at -80°C freezer.

Stability

DNaseMe is stable at -20°C for at least 3 years from the date of product release.
No loss in activity was observed after 12 months of incubation at RT.
No loss in activity was observed after 1 hour of incubation at 75°C.
DNaseMe does not lose its activity at least fifteen successive freeze/thaw cycles.

Shipping conditions

Shipping at room temperature or on blue/dry ice.

For research use only

Expiry

Information on the label

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