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Quick-Start Protocol

dsDNase

dsDNase (cat. no. EN33-050) is a 42.8 kDa recombinant endonuclease (dsDNase), 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. dsDNase is highly active in a broad spectrum of temperatures, buffer conditions and pH. The specific activity is similar to bovine DNase I however, dsDNase is characterized by higher stability in demanding reaction and storage conditions (e.g. high salt and detergent containing buffers, elevated temperatures). These features make dsDNase 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. The dsDNase must be shipped on dry ice and stored at –20°C in a freezer without a defrost cycle.

Notes before starting

- The dsDNase should be used for 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, and removal of residual DNA during primary stem cell isolation, biopharma and bioprocessing procedures.
- 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.

Considerations for use

1. The concentration of dsDNase is ≥ 20 U/µl.
2. The optimal concentration of dsDNase 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 dsDNase and incubation conditions have to be determined experimentally (we recommend using 35 U dsDNase for digestion of up to 50 µg dsDNA at 37°C for 10–15 min).
3. In the presence of Mg^{2+} or Ca^{2+} ions, dsDNase shows high endonuclease activity towards dsDNA leaving ssDNA and RNA essentially intact.
4. The presence of Ca^{2+} ions increases the total activity of dsDNase.
5. In the presence of Mn^{2+} ions, the enzyme shows slightly elevated dsDNase activity including activity towards ssDNA and RNA. Avoid using Mn^{2+} ions during purification of RNA or ssDNA.
6. Inactivation of dsDNase depends on the concentration of the reducing agent, inactivation time and temperature. We recommend inactivating dsDNase by incubation at 85°C for 15 min in the presence of reducing agents such as DTT or TCEP (1–10 mM).
7. The enzyme requires 1–10 mM DTT or TCEP to be completely inactivated.
8. Alternatively, dsDNase can be inactivated and removed by using a spin column or phenol/chloroform extraction.

Table 1. Operating conditions of dsDNase

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 temperatures)	0 – 100 mM
β-mercaptoethanol	0 – 2.5%

Document Revision History

Date	Changes
08/2023	Initial release

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