

Heat-Labile Double-Strand Specific DNase (HL-dsDNase)

- Double-strand DNA specific endonuclease
- Easily heat-inactivated by moderate heat treatment
- High specific activity
- Producing 5'-phospho-oligonucleotide products

“Double-strand DNA specificity - inactivated at low temperature”

Properties

Heat-Labile dsDNase is an endonuclease that cleaves phosphodiester bonds in DNA to yield oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. Heat-Labile dsDNase has a high specific activity, and it is easily inactivated by heat. It has a particularly strong preference for double-stranded DNA (dsDNA). In the presence of magnesium as only divalent cation and using oligos as a substrate; the activity towards dsDNA is minimum 5000-fold higher than towards ssDNA. The enzyme can therefore be used to specifically degrade dsDNA, leaving ssDNA essentially intact.

Activity determination: One Unit is defined as an increase in absorbance at 260 nm of 0.001 per minute, using 50 mg/ml high MW DNA in 50 mM Na-acetate pH 5.0 and 5 mM MgCl₂ (Kunitz, 1950).

Specific activity	Ca. 200 000 Kunitz Units/mg
pH optimum	pH 7.5 (in Tris-HCl)
Storage buffer	20 mM Tris-HCl pH 7.5, 2 mM MgCl ₂ , 10 mM NaCl, 0.01% (v/v) Triton X-100, 50% (v/v) glycerol
Reaction buffer	20 mM Tris-HCl pH 7.5, 5 mM MgCl ₂

Dependent on magnesium ions for activity.

Dependent on 1 mM DTT for inactivation.

Specificity towards double-stranded DNA

Table 1 Nuclease activity towards double- and single-stranded DNA oligonucleotides. Assay conditions: 25 mM Tris pH 7.5, 5 mM MgCl₂, and 2 μM oligonucleotide. The specificity of Heat-Labile dsDNase towards the substrate has been measured using a 15-mer oligonucleotide that is labelled 5'- with FAM and 3'- with DarkQuencher® (Eurogentec). The increase rate in fluorescence over time is directly proportional to enzyme activity.

Substrate	Relative activity	Compared to dsDNase activity
dsDNA	211 922	100%
ssDNA	10	0.004%

From the data above we can conclude that the Heat-Labile dsDNase is double-strand specific.

Heat inactivation

The Heat-Labile dsDNase can be heat inactivated by heat treatment at 55°C for 15 min. If proceeding directly to PCR, 55°C for 5 min is sufficient. The enzyme requires 1 mM DTT for inactivation.

Procedures

Removal of DNA from enzymes and mastermixes before PCR

1. Preincubate the mastermix without the template for 37°C for 10 min
2. Inactivate Heat-Labile dsDNase at 55°C, 15 min
3. Add template and run your PCR

RT-PCR

1. Add 0.1-0.5 U Heat-Labile dsDNase to your RT-PCR reaction
2. Incubate the reaction mix at 30°C for 15 min for DNA decontamination
3. Reverse transcription and inactivation of nuclease, 50°C for 30 minutes
4. Run your PCR

Closed-tube protocol!

Heat inactivation

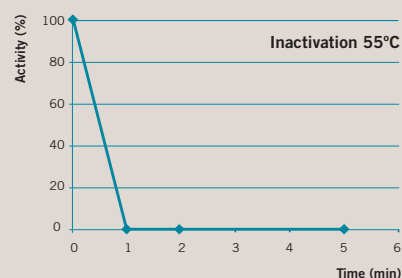


Figure 1 Residual activity of Heat-Labile dsDNase.

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