Double-Strand Specific DNase (dsDNase)

- Double-strand DNA specific endonuclease
- Can be heat-inactivated by moderate heat treatment
- High specific activity
- Producing 5'-phospo-oligonucleotide products

"Double-strand DNA specificity - inactivated at medium temperature"

Properties

dsDNase is an endonuclease that cleaves phosphodiester linkages in DNA to yield oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. dsDNase has a very high specific activity, estimated 30 times higher than bovine DNase I, and it is heat labile. dsDNase 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 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 $MgCl_2$ (Kunitz, 1950).

Specific activity	Ca. 475 000 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 8, 1-3 mM $MgCl_2$

Dependent on magnesium ions for activity.
Dependent on 1 mM DTT for inactivation.

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Specificity towards double-stranded DNA

Table 1 Nuclease activity towards double- and single-stranded DNA and RNA oligonucleotides. Assay conditions: 25 mM Tris pH 7.5, 5 mM $MgCl_2$, and 2 µM oligonucleotide. The specificity of 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	212 720	100%
ssDNA	55	0.026%
dsRNA	14	0.007%
ssRNA	13	0.006%

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

Heat inactivition

dsDNase can be heat inactivated by heat treatment at 65°C for 15 min.
We recommend to inactivate the dsDNase in the presence of 1 mM DTT.

dsDNase in one-step RT-PCR

Procedure

1. Add 0.1-0.5 U dsDNase to your RT-PCR reaction.
2. Incubate the reaction mix at 42°C for 15 min for DNA decontamination and reverse transcription.
3. Inactivate the dsDNase for 5 minutes at 95°C.
4. Run your PCR.

Closed-tube protocol!

Heat inactivation

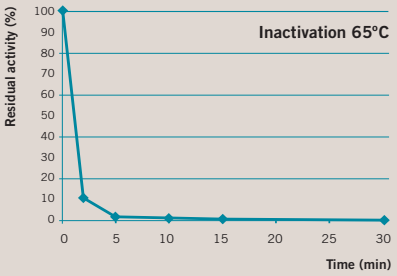

Figure 1 Residual activity of dsDNase. 60 Units dsDNase in 200 µl assay buffer was incubated at 65°C. Aliquots were taken out at indicated intervals and residual activity was measured.

References

Method for removal of contaminating PCR products from PCR or RT-PCR is subject to patent rights according to US patent 6,541,204 and equivalents. A license for using the patented method is conveyed by purchase of dsDNase from ArcticZymes AS or it's distributors.

Disclaimer

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