# Double-Strand Specific DNase (dsDNase)

- Double-strand DNA specific endonuclease
- Easily inactivated by heat
- No degradation of primers.

**"Rapid removal of contaminating DNA from PCR Mastermixes"**

## 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.

**Source:** Recombinantly produced in *Pichia pastoris.*

**Activity:** dsDNase is highly active in a temperature range of 20-40°C. It needs at least 2.5 mM Mg for activity and has an optimal pH at 7.5.

**Heat inactivation:** dsDNase is completely inactivated by incubating at 65°C for 15 min. 1 mM DTT is required for irreversible inactivation.

**Storage:** Minimum shelf life is 2 years at -20°C. Storage at 4°C is possible for at least 6 months. The enzyme also tolerates multiple freeze-thaw cycles.

**Purity:** dsDNase is purified to apparent homogeneity.
**Specific activity:** 470 000 Kunitz Unit/mg.

**Unit definition:** 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).

### Heat inactivation

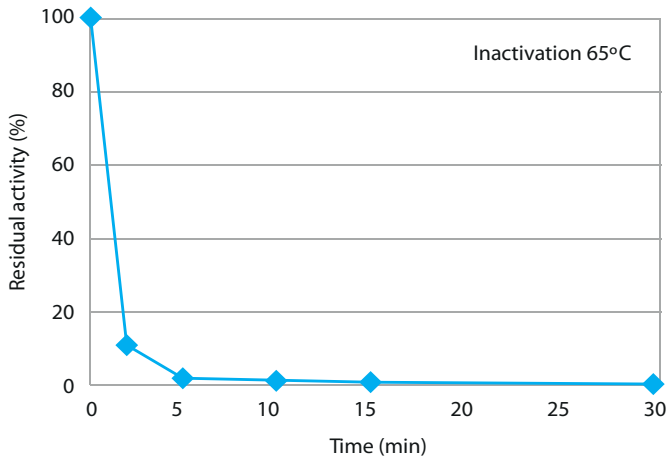


**Figure 1:** Residual activity of dsDNase. 60 Units dsDNase in 200 µl assay buffer was incubated at 65°C. Aliquots were taken out at indicated intervals and residual activity was measured.

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## dsDNase specificity

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®. The increase rate in fluorescence over time is directly proportional to enzyme activity. In table 1 we see the relative activity of dsDNase towards double-stranded and single-stranded DNA and RNA oligos.

dsDNase has a high specificity towards double-stranded DNA, leaving other nucleic acids unharmed.

| Substrate | Compared to dsDNase activity |
|---|---|
| dsDNA | 100% |
| ssDNA | < 0.03% |
| dsRNA | < 0.03% |
| ssRNA | < 0.03% |

**Table 1:** Nuclease activity towards double- and single-stranded DNA and RNA oligonucleotides.

## Decontamination of PCR mastermixes

Most Taq polymerases available are contaminated by bacterial DNA. This is a problem in PCR based bacterial typing and detection giving rise to false positive results. The unique properties of dsDNase make it very well suited for removal of contaminating DNA from PCR mastermixes prior to addition of DNA template. In figure 2 we have treated the PCR master mix with different amounts of dsDNase and used broad-range bacterial DNA specific primers to detect eventual contaminating bacterial DNA in the master mix. Only untreated PCR master mixes give a positive signal in Non-template-control qPCR.

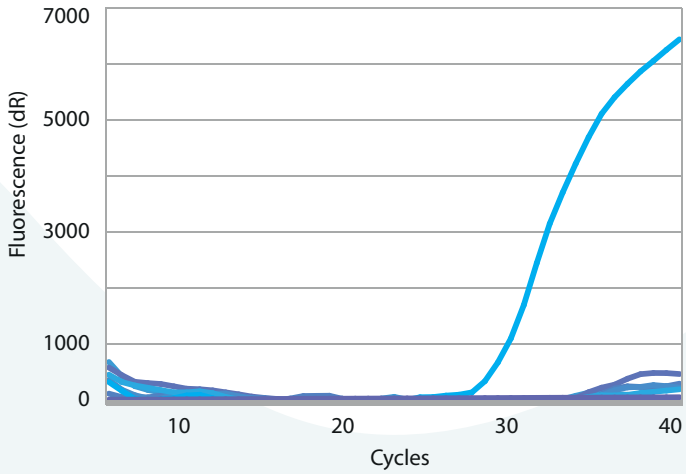
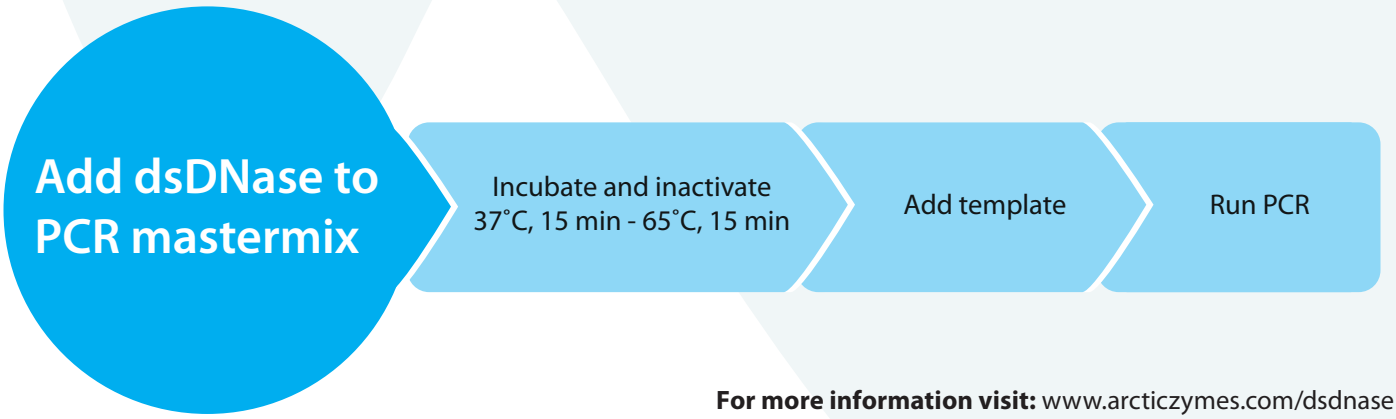



**Figure 2:** An optimal PCR buffer was preincubated with 0, 0.5, 1 or 5 Units of dsDNase. All dsDNase treatments resulted in complete removal of amplifiable DNA. Blue line: untreated reaction mix.

## Workflow - decontamination of PCR Mastermixes




**For more information visit:** www.arcticzymes.com/dsdnase





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