# ezDNase

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**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Product description

Invitrogen™ ezDNase™ Enzyme is a recombinant DNase for the fast removal of contaminating genomic DNA from RNA preparations. It eliminates double-stranded DNA specifically by cleaving phosphodiester bonds to yield 2–8 bp oligonucleotides with 5'-phosphate and 3'-hydroxyl termini.

The high specificity of ezDNase™ Enzyme for double-stranded DNA enables efficient and fast genomic DNA removal without reduction in the quality or quantity of RNA or single-stranded DNA present in the reaction such as cDNA and primers. ezDNase™ Enzyme is heat-labile and so can be easily deactivated by heat treatment at moderate temperature (55°C). These features make ezDNase™ Enzyme a superior choice for genomic DNA removal prior to reverse transcription reactions.

## Contents and storage

| Contents | Amount | Storage |
|---|---|---|
| ezDNase™ Enzyme | 50 µL | Store at –20°C |
| 10X ezDNase™ Enzyme Buffer | 100 µL | |
| Nuclease-free Water | 1.25 mL | |

## Source

The enzyme is purified from a *Pichia pastoris* strain expressing the recombinant DNase.

## Unit definition

One unit increases the absorbance at 260 nm by 0.001 OD per min at 25°C and pH 5.0 with large molecular weight DNA as the substrate according to the assay method of Kunitz.

## Storage buffer

The enzyme is supplied in 20 mM Tris-HCl (pH 7.5 at 25°C), 2 mM $MgCl_2$, 10 mM NaCl, 0.01% (v/v) Tween 20, 50% (v/v) glycerol.

## Inhibition and inactivation

- The enzyme is inhibited by metal chelators, transition metals (e.g., Zn) in millimolar concentrations, reducing agents (DTT and β-mercaptoethanol), SDS (even at concentrations <0.1%), and high salt concentration.
- Incubate at 55°C for 5 minutes in the presence of 10 mM DTT to inactivate the enzyme.

## Procedural guidelines

- Use RNase-free tubes, gloves and filter tips.
- If preparing several parallel reactions, use a master mix of water, buffer and ezDNase™ Enzyme to minimize the possibility of pipetting errors.

## Two-step gDNA digestion and RT PCR protocol

The following protocol allows gDNA digestion and cDNA synthesis to be combined in a two-step procedure within a single reaction tube. Ensure that the reaction volume does not exceed the allowable volume limits of the RT reaction.

1. Mix the following components in a RNase-free tube.

| Component | Volume |
|---|---|
| 10X ezDNase™ Enzyme Buffer | 1 µL |
| ezDNase™ Enzyme | 1 µL |
| Template RNA | up to 8 µL |
| Nuclease-free Water | to 10 µL |
| **Total volume** | **10 µL** |

2. Gently mix the samples then centrifuge the tube.
3. Incubate the sample for 2 minutes at 37°C.
4. (*Optional*) If the RNA sample is to be used for RT-PCR of fragments ≥3 kb, incubate the sample at 55°C for 5 minutes in the presence of 10 mM DTT to inactivate the enzyme.
5. Chill the tube on ice to bring the sample to room temperature, then briefly centrifuge and place the tube on ice.
6. Add reverse transcription reagents directly to the same tube and proceed with first strand cDNA synthesis protocol according to the manufacturer's instructions for your cDNA synthesis kit.

The product of the first strand cDNA synthesis can be used directly in PCR and qPCR or stored at –20°C for up to one week. For longer storage, –70°C is recommended. Avoid freeze/thaw cycles of cDNA.

## Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| PCR product is observed in the qPCR negative control reaction | The gDNA contamination was incompletely digested in the RNA sample. | Increase 37°C incubation step up to 5 minutes. |
| | Trace amounts of contaminants (SDS, EDTA, guanidine salts, phosphate, pyrophosphate, polyamines, spermidine) from the RNA purification process are inhibiting the DNase. | Re-precipitate the RNA with ethanol, wash the pellet with 75% ethanol then dissolve in nuclease-free water. |

## Limited product warranty

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