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Baseline-ZERO™ DNase

Cat. No. DB0715K

1. Introduction

Baseline-ZERO™ DNase* is ideal for use when you need to be certain that ZERO DNA remains. Baseline-ZERO DNase hydrolyzes both double-stranded (ds) and single-stranded (ss) DNA to mononucleotides with the highest efficiency (Fig. 1). In the presence of Mg^{2+}, cleavage of each strand of a dsDNA substrate proceeds independently.[1]

Baseline-ZERO DNase must be inactivated prior to the addition of Baseline-ZERO DNase-treated RNA to reverse transcription reactions. To inactivate the enzyme, incubate the completed reaction at 65°C for 10 minutes in the presence of 1X Stop Solution.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
Baseline-ZERO™ DNase	5,000 MBU	DB0715K	Baseline-ZERO™ DNase	E0110-D1	5 mL
			10X Baseline-Zero™ DNase Reaction Buffer	SS000751-D1	5 mL
			10X Baseline-Zero™ DNase Stop Solution	SS000752-D1	5 mL

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: Baseline-ZERO DNase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 10 mM $MgCl_2$, and 0.1% Triton® X-100.

Unit Definition: One Molecular Biology Unit (MBU) of Baseline-ZERO DNase produces an increase in the A_{260} of a solution of dsDNA, of 0.001 per minute at 25°C. Functionally, 1 MBU completely digests 1 µg of linear pUC19 DNA to mononucleotides in 10 minutes at 37°C.

10X Baseline-ZERO™ DNase Reaction Buffer: 100 mM Tris HCl (pH 7.5), 25 mM $MgCl_2$, and 5 mM $CaCl_2$.

10X Baseline-ZERO™ DNase Stop Solution: 30 mM EDTA.

Quality Control: Baseline-ZERO DNase is assayed for its ability to remove intact DNA and oligonucleotides from a Preparation of linear plasmid (Fig. 1).

Contaminating Activity Assays: Baseline-ZERO DNase is free of detectable RNase activities as assayed by PAGE analysis of 1 µg of a synthetic RNA transcript following an overnight incubation with enough Baseline-ZERO DNase to completely digest 1000 µg of DNA.

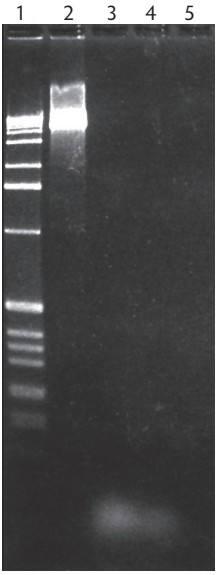
4. Applications

- Complete removal of DNA from RNA prior to RT-PCR.[2]
- Removal of ssDNA and dsDNA from viral RNA.
- Elimination of genomic DNA from RNA for microinjection and transfection experiments.
- Elimination of the DNA template following *in vitro* RNA synthesis with T7, T3, or SP6 Phage RNA Polymerases.

5. General Baseline-ZERO DNase Protocol

Note: *The reaction may be scaled up or down as needed.*

1. Resuspend the nucleic acid mixture (from any source) in 17 µL of RNase-Free water.
2. Add 2 µL of 10X Baseline-ZERO DNase Reaction Buffer to the sample.
3. Add 1 µL (1 MBU) of Baseline-ZERO DNase to the sample.
   Note: *1 MBU digests 1 µg of linear pUC19 DNA to dNMPs in 10 minutes at 37°C.*
4. Incubate at 37°C for 15-30 minutes.
5. Inactivate the Baseline-ZERO DNase by one of the following means.
   – Add 2 µL of 10X Baseline-ZERO DNase Stop Solution to the sample.
   – Incubate at 65°C for 10 minutes.
   or
   – Extract the sample with TE-saturated phenol/chloroform,
   – followed by a chloroform extraction
   – followed by a salt/ethanol precipitation.



Lane 1, Kilobase ladder

160 ng of linear plasmid DNA was incubated for 15 minutes at 37°C as follows:

Lane 2, untreated;

Lane 3, DNase I treated;

Lane 4, Hyperactive DNase treated (supplier A);

Lane 5, Baseline-ZERO DNase treated.

Figure 1. Baseline-ZERO™ DNase removes small oligonucleotides during DNase treatment.

Only Baseline™-ZERO DNase removes the small residual oligonucleotides visible at the bottom of the gel.

6. References

1. Sambrook, J. *et al.*, (1989) in: *Molecular Cloning: A Laboratory Manual (2nd ed.)*, Cold Spring Harbor Laboratory Press, New York.
2. Kienzle, N. *et al.*, (1996) *BioTechniques* **20**, 612.