PerfeCTa® DNase I (RNase-free)

Cat No. 95150-01K Size: 1000 units (2U/µL) **Store at -20°C**

Description

PerfeCta® DNase I is a high purity, recombinant bovine DNase I preparation that is free of any contaminating RNases or proteases. It provides a simple and rapid solution to eliminate residual genomic DNA from total RNA preparations for expression profiling by reverse transcription quantitative PCR amplification (RT-qPCR) as well as other molecular biology applications. The proprietary Reaction Buffer and Stop Buffer support a simple heat-kill step that permanently inactivates all trace levels of DNase activity. Complete inactivation of DNase I is critical before subsequent cDNA synthesis. Residual, or renatured, DNase will degrade cDNA product and thereby alter apparent expression levels.

One unit completely degrades 1µg of dsDNA in 10 minutes at 37°C.

Components

Name	Description	Amount
PerfeCta® DNase I	2 U/µL, recombinant DNase I, RNase-free in, 50 mM glycine (pH 7.2), 5 mM calcium acetate, 50%(v/v) glycerol and stabilizers.	0.5 mL
10X Reaction Buffer	Optimized 10X reaction buffer for DNase I	1 mL
10X Stop Buffer	10X chelation solution to remove divalent cations and inactivate the DNase I.	1 mL

Storage and Stability

PerfeCta® DNase I is stable for 2 years when stored in a constant temperature freezer at -20°C. The enzyme showed no loss in functional performance after 20 cycles of freezing on dry ice and thawing on ice. However, we recommend that the number of freeze-thaw cycles be kept to a minimum. For convenience, the 10X Reaction Buffer and 10X Stop Buffer may be stored at 2-8°C to avoid thawing the buffers for each use.

Reaction Assembly

Place components on ice. Mix, and then briefly centrifuge to collect contents to the bottom of the tube before using.

Component	Volume for 10-µL rxn.	Final Concentration
RNA template	variable	(up to 10 µg total RNA)
10X Reaction Buffer	1	1X
PerfeCta® DNase I (2 U/uL)	0.5 to 1	0.1 to 0.2 U/uL
RNase/DNase-free water	variable	
Total Volume (µL)	10 µL	

Note: for larger reaction volumes, scale components proportionally.

Reaction Protocol

- Combine reagents in 0.2-mL micro-tubes or 96-well plate sitting on ice.
- After sealing or capping each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.
- Incubate 30 min at 37°C
- Add 1 µL of 10X Stop Buffer.

  NOTE: Accurate pipetting of the Stop Buffer is critical! It is essential to chelate all divalent cations in the reaction for effective DNase I inactivation and to protect the RNA from chemical scission during the heat-kill step. Excess stop buffer can adversely affect downstream cDNA synthesis procedures.
- Vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.
- Incubate 10 min at 65°C
- Up to the entire reaction volume can be directly used as template for first-strand cDNA synthesis. Input quantity of DNase-treated total RNA should be adjusted as required for any given application.

Related Products

qScript™ cDNA SuperMix,	Cat. Nos. 95048-025, 95048-100, 95048-500
qScript cDNA Synthesis Kit,	Cat Nos. 95047-025, 95047-100, 95047-500
qScrtipt Flex cDNA Synthesis Kit,	Cat Nos. 95049-025, 95049-100

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