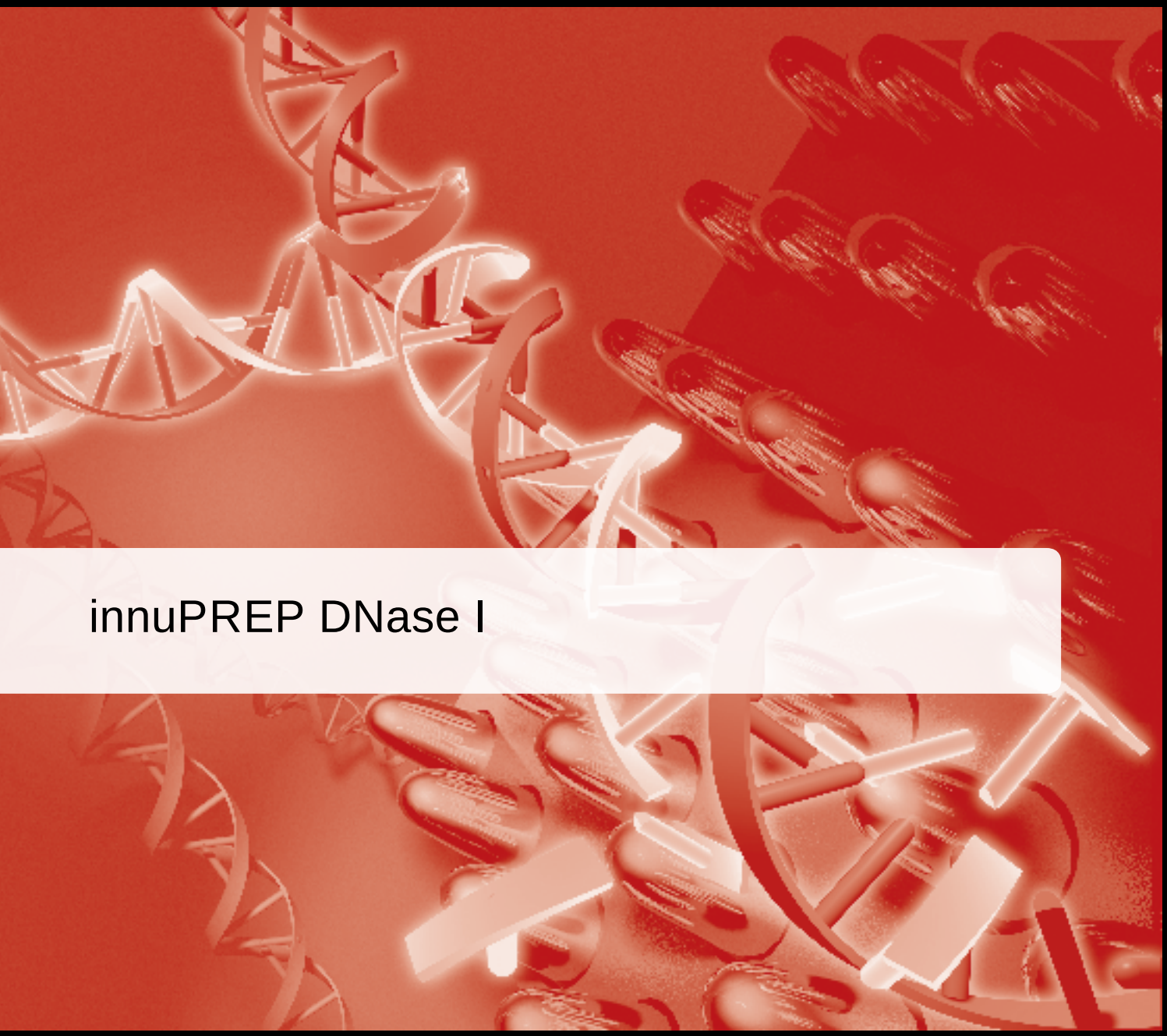
analytikjena

Manual



innuPREP DNase I

Life Science unlimited

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1 Product and order number

Name	Amount	Order-no.
innuPREP DNase I	5.000 Units	845-KS-5210005
innuPREP DNase I	10.000 Units	845-KS-5210010

2 Storage conditions

Store innuPREP DNase I at -20 °C in a freezer with constant temperature conditions.

If stored as recommended the innuPREP DNase I will remain stable at least for 12 months.

3 Description

The innuPREP DNase I has been developed for efficient digestion of DNA. For use whenever the presence of small quantities of contaminating DNA may affect the performance of assays, including RT-PCR – innuPREP DNAse I demonstrates high nuclease and is certified free from RNase activity.

innuPREP DNase I is an endonuclease which causes breaks of single- and double-stranded DNA resulting mono and oligo nucleotides. DNase I has no influence to RNA which will remain intact. The enzyme is used to remove unmeant co-purified genomic DNA from RNA preparations.

innuPREP DNase I acts independently of DNA sequence to cleave single-stranded, double-stranded DNA and chromatin to produce 5'-phosphate terminated polynucleotides with a 3' positioned free hydroxyl group.

3.1 Unit definition

One Kunitz unit is the amount of enzyme which is needed for increase of absorption (A260 nm) by 0.001 per minute and milliliter at 25 °C and pH 5.0.

3.2 Quality control

No RNase activity. No Inhibitors.

Functionality tested by digesting template DNA.

4 Delivered components

4.1 innuPREP DNase I

Concentration: 1 Kunitz Units/µl

DNase I in storage buffer 

The enzyme is supplied in:

50 mM Tris-HCl (pH 7,5), 10 mM $CaCl_2$ and 50% glycerol.

4.2 buffers

The innuPREP DNase I (1 KU/µl) is provided with the following buffers:

DNase I Reaction Buffer (10x) 

100 mM Tris-HCl (pH 7.5 at 25 °C), 25 mM $MgCl_2$ and 1 mM $CaCl_2$.

EDTA Solution 

50 mM EDTA.

5 Protocol

- Recommended protocols for digestion in combination with the:
  - innuPREP RNA Mini Kit
  - innuPREP RNA MIDI Direct Kit
  - innuPREP Blood RNA Kit
  - innuPREP Blood RNA MIDI Direct Kit
  - innuPREP Plant RNA Kit
  - innuPREP Micro RNA Kit
  - innuSPEED Tissue RNA Kit
  - innuSPEED Plant RNA Kit
  - innuPREP total RNA Kit

- After purification of the nucleic acid it could necessary to perform a DNase I step with the eluates of nucleic acids.

5.1 Removal of DNA from RNA preparations

- Prepare the following reaction mix in a DNase-/RNase-free reaction tube:

1 µl	innuPREP DNase I (1 Kunitz Units/µl) / 1 µg RNA
x µl	DNase I Reaction Buffer (10x)
add	with RNase-free water to the final reaction volume

- Incubate the reaction mix for 30 min at 37 °C.
- To inactivate the enzyme add 1 µl 50 mM EDTA or EGTA per 10 µl reaction mix and incubate the mixture for 10 min at 65 °C.

5.2 Ethanol precipitation

- Depending on the downstream application an ethanol precipitation can be reasonable:
- Therefore add 2.5 x volume 100 % Ethanol (pre-cooled at -20 °C) to the RNA solution, mix thoroughly and incubate the mix for minimum 10 min at -20 °C.
- Centrifuge the mixture and remove the supernatant and wash the RNA pellet once with 70 % ethanol by vortexing and subsequent centrifugation for 8 minutes at 7.500 x g (4 °C).
- At the end of the procedure briefly air-dry the pellet for 5 minutes. Dissolve the RNA pellet in DEPCdH2O or RNase-free buffer by vortexing or by passing it a few times through a pipette tip.

5.3 Hints and notes

- Don’t vortex DNase I, mix only by inverting of the tube carefully.
- Use only freshly prepared reaction mix.

6 Related products

Product	Amount	Order Number
innuPREP RNA Mini Kit	50 rxn	845-KS-2040050
	250 rxn	845-KS-2040250
innuPREP Blood RNA Kit	50 rxn	845-KS-2010050
	250 rxn	845-KS-2010250
innuPREP Plant RNA Kit	50 rxn	845-KS-2060050
	250 rxn	845-KS-2060250
innuPREP Micro RNA Kit	50 rxn	845-KS-2030050
	250 rxn	845-KS-2030250
innuPREP FFPE total RNA Kit	50 rxn	845-KS-2050050
	250 rxn	845-KS-2050250
innuSPEED Tissue RNA Kit	50 rxn	845-KS-2540050
	250 rxn	845-KS-2540250
innuSPEED Plant RNA Kit	50 rxn	845-KS-2560050
	250 rxn	845-KS-2560250

Publication No.: HB_KS-5210_e_151012

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

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