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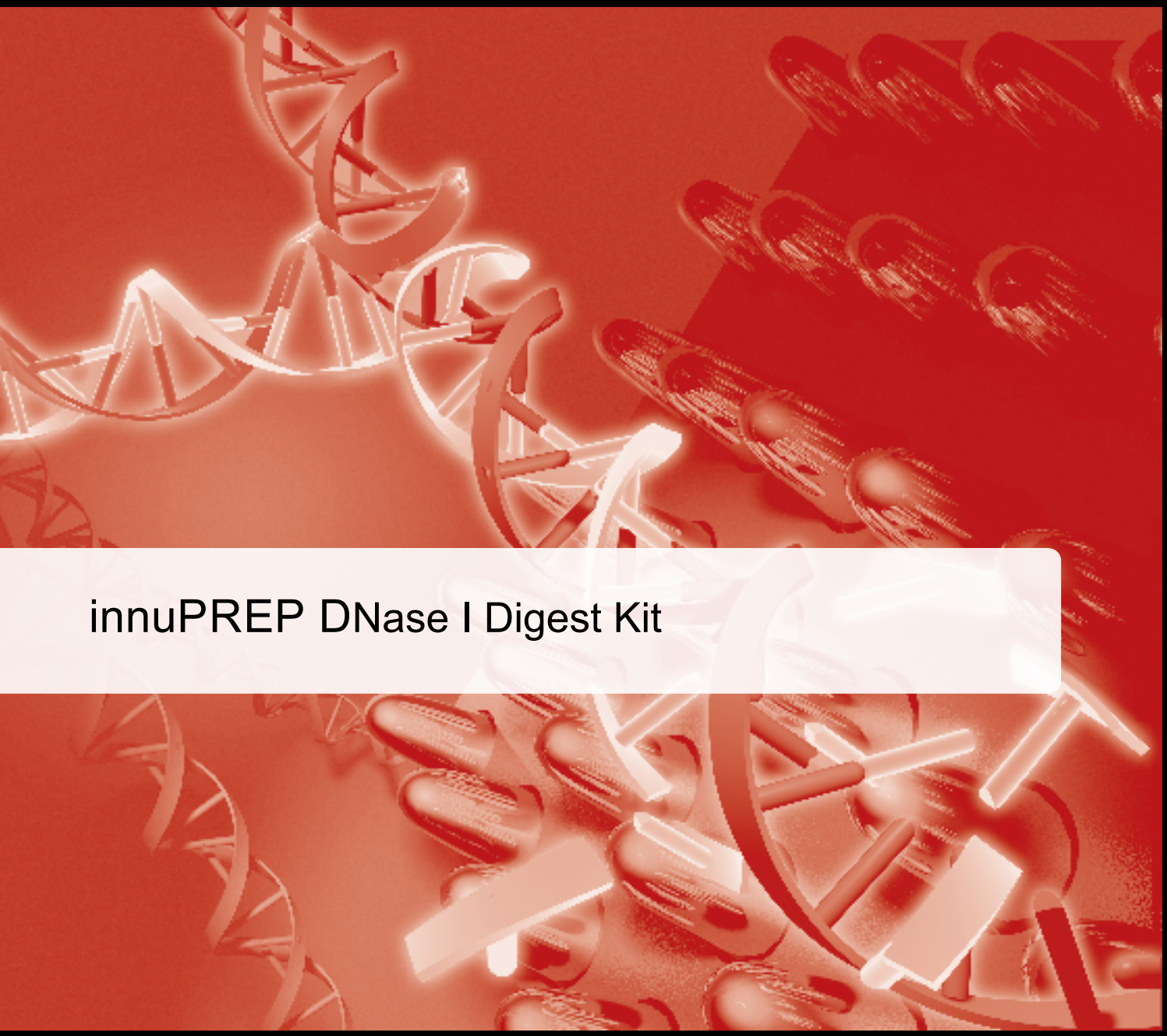
Manual



innuPREP DNase I Digest Kit

Life Science unlimited

Content

1 Product and order number

Name	Amount	Order-no.
innuPREP DNase I Digest Kit	10 rxn	845-KS-5200010
innuPREP DNase I Digest Kit	50 rxn	845-KS-5200050
innuPREP DNase I Digest Kit	250 rxn	845-KS-5200250

2 Storage conditions

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

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

3 Description

The innuPREP DNase I Digest Kit has been developed for efficient on-column digestion of DNA during RNA purification using kits based on binding of RNA on silica spin filter membranes. After lysis of starting material and subsequent binding of RNA on a spin filter the on-column digestion of DNA take place. After digestion of DNA the DNase I is removed in following washing steps.

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.

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 Important Note

For using the innuPREP DNase I Digest Kit in combination with other silica spin filter based RNA purification kits please read the specific information of the kit manual, respectively.

Normally, the on-column digestion step take place after binding of RNA on the spin filter following the subsequent washing steps.

4 Delivered components

4.1 innuPREP DNase I

Concentration: 20 Kunitz Units/µl

DNase I in storage buffer 

The enzyme is supplied in:

20 mM Tris-HCl (pH 7,5), 1 mM $MgCl_2$ and 50% glycerol.

4.2 buffer

The innuPREP DNase I Digest Kit (20 KU/µl) is provided with the following buffer:

DNase I Digestion Buffer 

25 mM Tris-HCl (pH 7.5 at 25 °C), 2.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$.

5 Protocol

- Recommended protocols for on-column digest 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 loading the homogenized and lysed sample onto the column (first steps of the Standard Protocol of RNA Kit) perform the optional DNase I step, *after loading and subsequent centrifugation of the sample onto the Spin Filter R*, as described below:

5.1 Step I

- Instead of performing the first washing step, add **300 µl Wasing Solution HS** onto the **Spin Filter R** located in a Receiver Tube and centrifuge for 1 minute at 10.000 x g.
- Discard the flow-throw liquid and reuse the Spin Filter R and Receiver Tube.

5.2 Step II

- Prepare the following reaction mix for each Spin Filter R:

innuPREP DNase I (20 Kunitz Units/µl))	1.5 µl
DNase I Digestion Buffer	73.5 µl
Total volume	**75.0 µl**

Important

- Don't vortex DNase I mixture. Mix only by inverting the tube.
- Use only freshly prepared DNase I mixture.
- Use only the DNase I Digestion Buffer that is supplied with innuPREP DNase I Digest Kit. Other standard DNase buffers are not compatible with this on-membrane DNase digestion.
- Don't use more starting material than recommended.
- The Lysis of the sample material should be completely.

- After preparing DNase I mixture apply 75 µl directly to the center of the membrane of the Spin Filter R.
- Incubate the Spin Filter R at room temperature (20 – 30 °C) for 15 minutes.

5.3 Step III

- After incubation place the Spin Filter R into a new Receiver Tube and add **300 µl Washing Solution HS**.
- Wait at least 5 minutes before proceeding.
- Centrifuge at 10.000 x g for 1 minute.
- Then continue with the washing step with Washing Solution LS according to the protocol. Finally the pure RNA can be eluted.

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

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