


# Instructions for Use

## Life Science Kits & Assays

A glass pipette is shown splashing water. Inside the pipette, a blue DNA double helix is visible. The background is a gradient of blue and black.

**innuPREP DNase I Digest Kit**

## 1 Product specifications

### 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 on RNA which will remain intact. The enzyme is used to remove unmeant co-purified genomic DNA from RNA preparations.

### Unit definition

One Kunitz unit is the amount of enzyme which is needed for increase of absorption ( $A_{260\text{ nm}}$ ) by 0.001 per minute and milliliter at 25 °C and pH 5.0.



### 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

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## Delivered components

Components		Description
innuPREP DNase I		Concentration: 20 Kunitz Units/ $\mu$ l
DNase I Digestion Buffer		25 mM Tris-HCl (pH 7.5), 2,5 mM MgCl <sub>2</sub> 0,5 mM CaCl <sub>2</sub>

## 2 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

## 3 Storage conditions

innuPREP DNase I Digest Kit is delivered at ambient temperature.

Store innuPREP DNase I Digest Kit at -22 to -18 °C in a freezer with constant temperature conditions.

When stored as recommended, the innuPREP DNase I Digest Kit is stable until the expiration date printed on the label on the kit box.

## 4 Safety precautions

The assay shall only be handled by educated personal in a laboratory environment. The compliance with the specified procedure is absolutely mandatory when performing this assay.

Reagents should be stored in their original containers at the indicated temperatures. Do not replace individual components with those from different batches or test assays. Note the indicated expiration dates.

Do not eat, drink or smoke while performing the assay.

Wear protective clothing and safety gloves.

All samples and test materials should be handled and disposed of as infectious material, in accordance with regulatory requirements.

Reagent containers that have not come in contact with potentially infectious material may be disposed of along with ordinary laboratory waste.

Store the reagents used for performing PCR separately from DNA templates and amplification products.

## 5 Protocol

Recommended protocols for on-column digest in combination with the IST Innuscreen RNA isolation kits (see related products)

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 200 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:

### 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)

Reagent	Volume (1 rxn)
innuPREP DNase I (20 Kunitz Units/µl)	1,5 µl
DNase I Digestion Buffer	73,5 µl
Total volume	75 µl

for 15 minutes.

### 5.3 Step III

- After incubation place the Spin Filter R into a new Receiver Tube and add 300  $\mu$ l 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	Order Number
innuPREP RNA Mini Kit 2.0	845-KS-2040050
	845-KS-2040250
innuPREP Blood RNA Kit	845-KS-2010050
	845-KS-2010250
innuPREP Plant RNA Kit	845-KS-2060050
	845-KS-2060250
innuPREP Micro RNA Kit	845-KS-2030050
	845-KS-2030250
innuPREP FFPE total RNA Kit	845-KS-2050050
	845-KS-2050250

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