400 Pinnacle Way, Ste 450
Norcross, GA 30071
USA
Tel: 770-931-8400
Fax: 770-931-0230

RNase-Free DNase I Set

The E.Z.N.A. RNase-Free DNase I Set is optimized for use with E.Z.N.A.® Total RNA protocols. Normally DNase I digestion is not required for RNA purified with HiBind® RNA Mini Columns as our silica-based spin column technology efficiently removes the majority of DNA without enzymatic digestion. However, certain sensitive RNA applications may require further DNA removal.

Activity: 10,000 Kunitz units/mg

One Kunitz unit is defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25 °C, pH 5.0, with highly polymerized DNA as the substrate (1).

Reaction Time: 15-20 minutes on column at 20-30 °C

Concentration: 20 Kunitz/µL

Storage/Stability: Store at -20°C. All components of the RNase-Free DNase I Set are stable for at least 24 months from the date of purchase when stored at -20°C.

Format: Lyophilized enzyme with RNase-free buffer and water

Kit Contents

Product	Preps	Units of DNase I
E1091-00	5	180
E1091-01	50	1,500
E1091-02	200	6,000

On-membrane DNase I Digestion Protocol

The following protocol is a short procedure for On-Membrane DNase I digestion. Please take a few minutes to read the user manual accompanying the E.Z.N.A.® RNA Kit thoroughly to become familiar with the protocol. Prepare all materials required before starting the RNA isolation procedure to minimize RNA degradation. Follow the standard E.Z.N.A® RNA protocol until the optional step for on-membrane DNase I digestion.

1. For each HiBind® RNA Mini Column, prepare the DNase I stock solution as follows:

Buffer	Volume per Prep
E.Z.N.A.® DNase I Digestion Buffer	73.5 µL
RNase-free DNase I (20 Kunitz/µL)	1.5 µL
Total Volume	75 µL

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. **Do not vortex the DNase I mixture.** Mix gently by inverting the tube.
- Freshly prepare DNase I stock solution right before RNA isolation.
- Standard DNase buffers are not compatible with on-membrane DNase I digestion. The use of other buffers may affect the binding of RNA to the HiBind® matrix and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

2. Insert the HiBind® RNA Mini Column containing the sample into a 2 mL Collection Tube.

3. Add 1/2 volume RNA Wash Buffer I (compared to the standard E.Z.N.A.® RNA protocol) to the HiBind® RNA Mini Column.

4. Centrifuge at 10,000 x *g* for 1 minute.

5. Discard the filtrate and reuse the Collection Tube.

6. Add 75 µL DNase I digestion mixture directly onto the surface of the membrane of the HiBind® RNA Mini Column.

   Note: Pipet the DNase I directly onto the membrane. DNA digestion will not be complete if some of the mixture is retained on the wall of the HiBind® RNA Mini Column.

7. Let sit at room temperature for 15 minutes.

8. Add 1/2 volume RNA Wash Buffer I (compared to the standard E.Z.N.A.® RNA protocol) to the HiBind® RNA Mini Column.

9. Continue to the RNA Wash Buffer II wash step in the standard E.Z.N.A.® RNA protocol.