Product Information Sheet and Protocol

Product Name: EB Pure DNase I Digestion Kit*
Concentration: 20 Kunitz/uL
Item No.:

Item Number	Preparations
EB-DDK-50	50
EB-DDK-200	200

Storage and Handling:
Store at -20°C upon arrival. DNase Digestion Buffer can be stored at room temperature or -20°C.

Product Description:
EB Pure DNase I Digestion Kit is optimized for use with EB Pure Total RNA Kit protocol. Normally DNase I digestion is not required for RNA purified with High Bind RNA Mini Columns which efficiently remove the majority of DNA without enzymatic digestion. However, certain sensitive RNA applications may require further DNA removal.

Kit contents:

Component	EB-DDK-50	EB-DDK-200
DNase Digestion Buffer	1mL	5mL
DNase I (20 Kunitz/uL)	10uL	78uL
Units of DNase I	1,500	6,000

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 EC, pH 5.0, with highly polymerized DNA as the substrate.

Reaction Time:
15-20 minutes on column at 20-30 EC.

* This product is for “Research Use Only. Not for use in diagnostic procedures”.
For MSDS and Certificate of Analysis please visit www.empiricalbioscience.com

Protocol and Preparation Procedure:

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 EB Pure Total 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 EB Pure Total RNA protocol until the optional step for on-membrane DNA I digestion.

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

Buffer	Volume per Prep
DNase Digestion Buffer	73.5 uL
DNase I (20 Kunitz/uL)	1.5 uL
Total Volume	75 uL

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 matrix and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

2. Insert the High Bind RNA Mini Column containing the sample into a 2mL Collection Tube.
3. Add ½ volume of RNA Wash Buffer I (compared to the standard EB Pure Total RNA protocol) to the High Bind RNA Mini Column.
4. Centrifuge at 10,000 x g for 1 minute.
5. Discard the filtrate and reuse the 2mL Collection Tube.
6. Add 75uL DNase I digestion mixture directly onto the surface of the membrane of the High Bind 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 High Bind RNA Mini Column.
7. Let sit at room temperature for 15 minutes.
8. Add ½ volume of RNA Wash Buffer I (compared to the standard EB Pure Total RNA protocol) to the High Bind RNA Mini Column.
9. Continue to the RNA Wash Buffer II wash step in the standard EB Pure Total RNA protocol.