RNASE A, LYOPHILIZED

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-NUC-007
Product Name	RNase A, Lyophilized
Category	Ribonuclease
Pack Size	1g/bottle, 10g/bottle
Regulatory Status	For Research Use Only (RUO)
OEM Reference	GPE007001
Version	1.0
Issue Date	2026-05-14

2. INTENDED USE

RNase A is a recombinant bovine pancreatic endoribonuclease (13.7 kDa) that specifically cleaves single-stranded RNA at the 3'-phosphate group of pyrimidine nucleotides (cytosine and uracil). This molecular biology grade enzyme (≥40 Kunitz units/mg protein, DNase-free) is used to remove RNA contamination from plasmid and genomic DNA preparations, eliminate RNA from protein samples, perform RNase protection assays, analyze RNA sequences, and map single-base mutations in DNA or RNA. The recombinant preparation offers superior lot-to-lot consistency and purity compared to native pancreatic RNase A, with optimal activity at pH 7.6 and 60°C, remaining active across pH 6-10 and temperatures of 15-70°C. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
RNase A, Lyophilized Powder	1 g (≥40 Kunitz units/mg protein)	Store at -20°C
RNase A, Lyophilized Powder	10 g (≥40 Kunitz units/mg protein)	Store at -20°C

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Appropriate buffer solution (e.g., 10 mM Tris-HCl, pH 7.5-8.0) for reconstitution of lyophilized powder
- Nuclease-free water for reconstitution and dilution
- RNA substrate (single-stranded RNA or yeast RNA) for enzymatic activity
- DNA or RNA sample requiring RNase treatment
- Appropriate microcentrifuge tubes (RNase-free, siliconized or low-binding recommended due to high affinity to glass)
- Temperature-controlled incubator or water bath capable of maintaining 15-70°C (optimal activity at 60°C)
- Spectrophotometer for activity measurement at 260 nm
- RNase inhibitor (optional, such as RIBOPROTECT or equivalent) for controlled digestion reactions

5. STORAGE AND STABILITY

Storage Temperature	-20°C
Appearance	Light yellow or white lyophilized powder
Shelf Life	12 months from manufacture date
Shipping Conditions	On dry ice
Freeze-Thaw Cycles	Maximum 3 cycles recommended
Working Solution	Stable on ice for up to 8 hours

6. PRECAUTIONS AND WARNINGS

- For Research Use Only. Not for use in diagnostic procedures.
- Avoid repeated freeze-thaw cycles. Aliquot reagents if needed.
- Handle all reagents on ice. Return to -20°C storage immediately after use.
- Wear appropriate PPE: gloves, lab coat, and eye protection at all times.
- Dispose of waste in accordance with local, state, and federal regulations.
- Do not use reagents past their expiry date.

7. PROTOCOL

DETAILED PROTOCOL FOR RNase A, LYOPHILIZED

ENZOVERA LIFE SCIENCES

PRODUCT: RNase A (Recombinant Bovine Pancreatic Ribonuclease)

CATALOG NUMBER: Contact Enzovera Technical Support at techsupport@enzovera.com

SPECIFIC ACTIVITY: ≥40 Kunitz Units/mg protein

MOLECULAR WEIGHT: 13.7 kDa

INTENDED APPLICATIONS

RNase A is an endoribonuclease that specifically cleaves single-stranded RNA at the phosphodiester bond between the 5'-ribose of a nucleotide and the 3'-phosphate group of an adjacent pyrimidine nucleotide. This enzyme is used for:

- Removal of RNA contamination from plasmid DNA preparations

- Removal of RNA from genomic DNA preparations

- Removal of RNA from protein samples

- RNase protection assays

- RNA sequence analysis

- Mapping single-base mutations in DNA or RNA

PRODUCT SPECIFICATIONS

Source: Recombinant expression in eukaryotic cells (Saccharomyces cerevisiae) expressing cloned bovine pancreatic ribonuclease gene

Purity: Molecular biology grade; endonuclease contamination none detected; exonuclease contamination none detected; free of DNase activity

Form: Lyophilized powder

Optimal Temperature: 60°C (active range 15-70°C)

pH Range: Active pH 6-10; optimal pH 7.6

Storage: -20°C

RECONSTITUTION OF LYOPHILIZED RNase A

1. Remove the vial of lyophilized RNase A from -20°C storage and allow to equilibrate to room temperature (20-25°C) for 5 minutes before opening to prevent condensation.

2. Briefly centrifuge the vial at 1000 x g for 10 seconds to collect lyophilized powder at the bottom of the tube.

3. Add sterile nuclease-free water or 10 mM Tris-HCl, pH 8.0 to achieve the desired concentration. For a 10 mg/mL stock solution, add 1 mL of buffer per 10 mg of lyophilized enzyme.

4. Gently mix by pipetting up and down 10 times or by gentle vortexing for 5 seconds. Do not vortex vigorously as this may denature the enzyme.

5. Incubate at room temperature for 5-10 minutes with occasional gentle mixing to ensure complete dissolution.

6. Centrifuge the reconstituted solution at 10,000 x g for 1 minute at 4°C to pellet any insoluble material.

7. Transfer the supernatant to a fresh nuclease-free tube. The reconstituted enzyme is now ready for use.

8. Note: At neutral pH and high concentrations greater than 10 mg/mL, the enzyme may precipitate. If precipitation occurs, dilute the solution with 10 mM Tris-HCl, pH 8.0 to achieve a final concentration of 5-10 mg/mL.

PROTOCOL 1: REMOVAL OF RNA FROM PLASMID DNA PREPARATIONS

This protocol describes the use of RNase A to remove contaminating RNA from plasmid DNA prepared by alkaline lysis or other methods.

1. Prepare a working solution of RNase A at 10 µg/mL in 10 mM Tris-HCl, pH 8.0 by diluting the reconstituted stock solution.

2. Add RNase A to the cleared plasmid lysate to achieve a final concentration of 10 µg/mL. For example, add 10 µL of 10 µg/mL RNase A solution per 1 mL of lysate.

3. Mix gently by inversion 5 times or by gentle pipetting.

4. Incubate the mixture at 37°C for 15-30 minutes to allow complete RNA digestion.

5. Note: No heating step is required before use as this recombinant RNase A is DNase-free.

6. Proceed with standard plasmid DNA purification methods such as phenol-chloroform extraction and ethanol precipitation, or use spin column purification to remove the enzyme and RNA fragments.

7. For spin column purification, follow the manufacturer's protocol for DNA cleanup. The digested RNA fragments and RNase A will be removed during the wash steps.

8. For phenol-chloroform extraction, add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to the sample, vortex for 30 seconds, and centrifuge at 12,000 x g for 5 minutes at room temperature. Transfer the aqueous phase to a fresh tube. Repeat extraction 2-3 times to ensure complete removal of RNase A.

9. Precipitate the plasmid DNA by adding 0.1 volumes of 3 M sodium acetate, pH 5.2 and 2.5 volumes of ice-cold 100% ethanol. Mix by inversion and incubate at -20°C for 30 minutes.

10. Centrifuge at 12,000 x g for 15 minutes at 4°C to pellet the DNA. Discard the supernatant.

11. Wash the DNA pellet with 1 mL of ice-cold 70% ethanol and centrifuge at 12,000 x g for 5 minutes at 4°C.

12. Carefully remove the supernatant and air-dry the pellet for 5-10 minutes at room temperature.

13. Resuspend the purified plasmid DNA in sterile nuclease-free water or 10 mM Tris-HCl, pH 8.0.

14. Verify RNA

8. EXPECTED RESULTS

When RNase A, Lyophilized is reconstituted and applied to RNA-contaminated plasmid DNA preparations or protein samples, complete degradation of single-stranded RNA should be observed within 15–30 minutes at room temperature or 37°C, as confirmed by agarose gel electrophoresis showing elimination of RNA bands while DNA remains intact. The enzyme exhibits maximum activity at 60°C and pH 7.6, with specific cleavage at C↓p and U↓p sites (3' to pyrimidine residues). Properly stored enzyme at -20°C maintains ≥40 Kunitz units/mg protein specific activity and shows no detectable endonuclease, exonuclease, or DNase contamination.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

Document Number	IFU-EV-NUC-007
Version	1.0
Status	DRAFT — Pending Authorisation
Issue Date	2026-05-14
Prepared By	Enzovera Life Sciences Technical Documentation
Approved By	Pending
Next Review	12 months from issue date