GB RNase A **For Research use only**

Cat No: GB2443

Size : 1 gr

Description:

RNase A is an endoribonuclease that attacks at the phosphate of a pyrimidine nucleotide. The sequence of pG-pG-pCpA-pG will be cleaved to give pG-pG-pCp and A-pG. The highest activity is exhibited with single stranded RNA. RNase A is a single chain polypeptide containing disulfide bridges. Activators of RNase A include potassium and sodium salts.

Applications of RNase A

1. **Plasmid and Genomic DNA Preparation**:
   - **Purpose**: To remove contaminating RNA from DNA preparations.
   - **Protocol**:
     - Add RNase A to the lysate during the alkaline lysis step of plasmid DNA preparation.
     - Incubate at 37°C for 30 minutes to allow RNase A to degrade RNA.
2. **Protein Purification**:
   - **Purpose**: To remove RNA contaminants in recombinant protein preparations.
   - **Protocol**:
     - Add RNase A to the protein solution at a final concentration of 10-100 µg/mL.
     - Incubate at 37°C for 30 minutes.
3. **Ribonuclease Protection Assays**:
   - **Purpose**: To map single-base mutations or to quantify specific RNAs in a sample.
   - **Protocol**:
     - Hybridize labeled RNA probes to the target RNA.
     - Treat with RNase A to degrade unprotected regions.
     - Analyze protected fragments by gel electrophoresis.

Preparation:

Solutions prepared from powdered RNase A products can be made free of DNase by boiling. According to one literature method:

1. Prepare a 10 mg/mL stock solution in 10 mM sodium acetate buffer, pH 5.2.

2. Heat to 100°C for 15 minutes. Allow to cool to room temperature. 3. Adjust to pH 7.4 using 0.1 volume of 1 M Tris -HCl, pH 7.4 (i.e. add 500 µl 1M Tris-HCl, pH 7.4 to 5 ml of 10 mg/ml RNase stock solution).

4. Aliquot and store at –20°C. If RNase A is boiled at a neutral pH, precipitation will occur. When boiled at the lower pH, some precipitation may occur because of protein impurities that are present.

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Or Stock solutions are prepared at concentrations from 1 - 10 mg/ml in 10 mM Tris HCl (pH 7.5); 15 mM NaCl or in Tris HCl (pH7.5); 1 mM EDTA, pH 8 (TE buffer). The recommended working concentration is 10 µg/ml(removal from RNA from plasmid preparations; 1 hr, RT) or 100 ng/ml (preparation of 'blunt ends' of double-stranded cDNA).

How to use:

1- Add RNase A to your sample to achieve the desired final concentration (typically 10-100 µg/mL).
2- Incubate the sample at 37°C for 30 minutes to 1 hour, depending on the amount of RNA to be degraded.
3- Inactivate RNase A (if necessary): If further downstream applications require the removal of RNase A, you can inactivate it by adding proteinase K and incubating at 37°C, followed by phenol-chloroform extraction and ethanol precipitation.

Safety and Handling

- **Toxicity**: RNase A is generally considered safe, but it should be handled with care to avoid inhalation or skin contact.
- **Storage**: RNase A can be stored at -20°C or lower for long-term storage. For short-term use, it can be kept at 4°C.
- **Contamination**: Ensure all glassware and buffers used with RNase A are RNase-free to prevent any contamination of RNA samples.

Important Notes:

- **Handling RNase A**: RNase A is a stable enzyme and resistant to boiling and autoclaving. However, always handle it with care to avoid contamination of RNA-sensitive samples.
- **DNase Contamination**: Ensure RNase A solutions are free from DNase contamination by boiling the solution as described.
- **RNA Removal**: This protocol is effective for removing RNA contaminants in plasmid and genomic DNA preparations.

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