Protocol

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DNase Inactivation in RNase A Solution Protocol

Introduction

RNase is an endonuclease that cleaves single-stranded RNA, and is commonly used to eliminate carry over RNA in various procedures including plasmid purification. The presence of DNase, an endonuclease that cleaves DNA, in an RNase solution, can cause the unintended breakdown of DNA in biological samples. Thus, the inactivation of DNase is necessary. RNase can be rendered pure and DNase-free through a simple heat deactivation procedure. This allows for the controlled cleavage of RNA for further downstream applications including protection assays and RNA sequence analysis. This protocol describes the steps necessary to deactivate DNases in an RNase solution.

Materials

- RNase A (GoldBio Catalog # R-050)
- Sodium acetate buffer
- Tris-HCl (GoldBio Catalog # T-095)

Method

1. Prepare a 10 mg/ml stock solution of RNase A in 10mM Sodium Acetate buffer at a pH of 5.2.

2. Heat solution to 100°C for 15 minutes.

3. Allow mixture to cool to room temperature, then adjust pH to 7.4 using a one tenth volume of 1M Tris-HCl at a pH of 7.4 (i.e. add 500 µl 1M Tris-HCl, pH 7.5 to 5 ml of 10 mg/ml RNase stock solution).

4. Aliquot into individual use tubes and store at -20°C. RNase is stable for up to 2 years when stored properly at -20°C.

Note: If RNase A is boiled at a neutral pH, then precipitation of RNase A will occur. When boiled at a lower pH, some precipitation may occur, but this is because of protein impurities that may exist and not the RNase A itself.

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Associated Products

- RNase A (GoldBio Catalog # R-050)
- Tris-HCl (GoldBio Catalog # T-095)

References

Boiled RNase A. (1970, January 01). Retrieved April 27, 2018, from http://cshprotocols.cshlp.org/content/2006/1/pdb.rec213.full?text_only=true.