In-house RNA stabilization solution

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Most molecular biology and diagnostic applications require the isolation and purification of high yields and quality of RNA. However, RNA is very unstable and prone to degradation before and during processing. Factors contributing to the degradation of RNA include enzymes such as RNAses, elevated temperature and high pH value. RNA is also susceptible to spontaneous hydrolysis under alkaline conditions leading to complete, sequence-independent degradation of RNA molecules to mononucleotides. Such hydrolysis reaction is driven by deprotonation of the 2'-OH group of the ribose under elevated OHH ion concentration aqueous solutions. RNA degradation is significantly inhibited in the presence of ammonium sulfate [either the preferred slat known as diammonium sulfate ($(NH_4)2S0_4$) or the alternate ammonium bisulfate (NH_4HS0_4)] in the presence of a chelating agent (EDTA).

RNAlater-like RNA stabilization solution is an aqueous, nontoxic tissue storage reagent capable of permeating tissues that stabilizes and protects cellular and/or viral RNA. This solution minimizes the need to immediately process a biospecimen or to transport these samples in liquid nitrogen for future processing. Tissue samples and biological liquids can be harvested and submerged in this solution for storage without jeopardizing the integrity, quality or quantity of the extracted RNA.

Preparation

1. Prepare 40 mL of 0.5 M EDTA by dissolving 7.44 g EDTA in 40 mL of dH_2O. Adjust pH to 8.0 with either HCL or NaOH.

2. Prepare 25 mL of 1M Trisodium Citrate by dissolving 5.88 g of Sodium Citrate in 25 mL of dH_2O.

3. Place a 1 litre flask on a magnetic stirrer set at 30ºC and mix 40 mL of 0.5 M EDTA and 25 mL of 1M Trisodium Citrate.

4. Dissolve 700 g of Ammonium Sulphate in the previous mix of EDTA and Trisodium Citrate and continue magnetic stirring until completely dissolved.

5. Top up to 1 litre with dH_2O, allow to cool and adjust pH to 5.2 using H_2SO_4.

6. Store at room temperature.

Notes

1. This RNA stabilization solution provides immediate protection from RNase activity, freedom from liquid nitrogen and freezers and is ideal for field specimen collection. It has been shown to be compatible with most RNA isolation and downstream procedures.

2. Final concentrations:
    a. 25 mM Sodium Citrate
    b. 10 mM EDTA
    c. 70 g ammonium sulphate/100 ml solution
    d. pH 5.2

References

1. Rebecca P. Duncan, University of Miami on "When making homemade RNAlater, how long should it take for the ammonium sulfate to dissolve?" ResearchGate December 13, 2013. https://www.researchgate.net/post/When-making-homemade-RNAlater-how-long-should-it-take-for-the-ammonium-sulfate-to-dissolve

2. Bock, R.M.: Alkaline hydrolysis of RNA, Methods in Enzymology, Volume 12, Part A, 1967, pages 224-228; Elliot, D., Ladomery, M.: Molecular Biology of RNA (1 st ed. 201 1). New York: Oxford University Press, pages 34-64).

Revision history

1.0 Original document.

2.0 Changes to document format only.

3.0 Corrected reference and provided final concentrations.

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