May 14, 2020

ONA and RNA stabilization

DOI

dx.doi.org/10.17504/protocols.io.be2pjgdn

Neilier Junior¹

¹University of Manitoba



Neilier Junior

University of Manitoba, Universidade Federal de Viçosa



DOI: <u>dx.doi.org/10.17504/protocols.io.be2pjgdn</u>

Protocol Citation: Neilier Junior 2020. DNA and RNA stabilization. protocols.io <u>https://dx.doi.org/10.17504/protocols.io.be2pjgdn</u>

License: This is an open access protocol distributed under the terms of the <u>Creative Commons Attribution License</u>, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

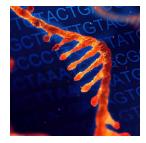
Protocol status: Working We use this protocol and it's working

Created: April 13, 2020

Last Modified: May 14, 2020

Protocol Integer ID: 35631

Keywords: DNA, RNA, RNA stabilization, Molecular Biology, Biochemistry, RNA extraction, RNase free, Nucleic acids, RNA-seq,



Abstract

DNA and RNA stabilization solution is an aqueous and non-toxic tissue storage reagent that rapidly permeates tissues to stabilize and protect nucleic acids in fresh specimens. Tissue pieces are harvested and immediately submerged in stabilization solution for storage. It can be used for DNA and RNA preservation with most tissues, cultured cells and is compatible with most DNA/RNA isolation methods. Proteins are precipitated, but can still be used for analyzes that do not require their native structure, such as western blotting or gel electrophoresis. Samples in DNA and RNA stabilization solution can be stored indefinitely in RNAlater Solution at -20 °C or below.

Guidelines

Use only RNase-free tubes, tips and flasks.

Materials

MATERIALS

X Ammonium Sulfate P212121

Sulfuric acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #258105

Sodium Citrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #71402

EDTA Thermo Fisher Catalog #17892

Use only RNase-free tubes, tips and flasks.

Safety warnings

• Wear personal protective equipment: gloves, clothing and mask.

Before start

Organize your workspace.

Make sure all solutions and equipment are available. Plan the experiment!

Stabilization solution preparation (1.5 L)

- 1
- 935 mL of autoclaved MilliQ water
- 700 g ammonium sulfate
- Stir until dissolved
- Add 25 mL of 1 M sodium citrate
- Add 40 mL of 0.5 M EDTA
- Adjust to pH 5.2 using concentrated H₂SO₄
- Complete the volume to 1.5 L with autoclaved MilliQ water

Note

- 1. Store at room temperature.
- 2. If any precipitation of stabilization solution is seen: heat to 37 °C and agitate to redissolve it.

Procedure

- Do not freeze tissues before immersion in DNA and RNA stabilization solution.
 - Use stabilization solution with fresh tissue only.

3

2

Add the stabilization solution in an appropriate 1.5 mL microtube.

Note

Use the minimum volume ratio 1:5 (tissue : solution).

- 4 Cut large tissue samples to approx. 3 mm in any single dimension and transfer to microtube containing the solution.
- 5 Store at 4 °C overnight to allow the solution to thoroughly penetrate the tissue.
- 6 **Do not freeze samples in stabilization solution immediately.**

Important notes

- 7 The DNase and RNase inactivation is reversible. So, do not rinse stabilization solution from samples before DNA and/or RNA extraction.
- 8 Tissue homogenization should be rapid once the tissue is in lysis/denaturation solution to nucleic acids extraction.
- 9 Remove the stabilization solution from the cells prior to extraction for isolating DNA and RNA from cells.

With the aid of a pipette, remove excess of stabilization solution from the tube and then use an absorbent lab wipe or paper towel to help remove as much of the stabilization solution as possible.