Edwards lab protocol for preparing RNAlater preservative salt solution

From Brant Faircloth's recipe and instructions at <gist.github.com/brantfaircloth/0229c3ae545f4bcfe4de>,
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What is RNAlater?

RNAlater is an aqueous, nontoxic tissue DNA and RNA stabilization and storage reagent that rapidly permeates tissues to stabilize and protect cellular DNA and RNA. RNAlater solution minimizes the need to immediately process tissue samples or to freeze samples in liquid nitrogen for later processing. Tissue pieces can be harvested and submerged in RNAlater solution for storage without jeopardizing the quality or quantity of DNA or RNA obtained after subsequent nucleic acid isolation.

How do I use RNAlater solution?

Cut up the tissue to be less than 0.5 cm in at least one dimension and simply submerge it in 5 volumes of RNAlater solution (e.g., a 0.5 g sample requires about 2.5 mL of RNAlater solution). Small organs can be stored whole in RNAlater solution. If preserving cells, resuspend pelleted cells in a small amount of PBS before adding 5–10 volumes of RNAlater solution. Samples can be stored at 4°C for one month, at 25°C for one week, or at –20°C indefinitely. Archive tissues treated with RNAlater solution at –20°C or colder.

For DNA or RNA isolation, remove the tissue from RNAlater solution and treat it as though it was just harvested. Most tissues can be homogenized directly in lysis buffer, although harder tissues such as bone may require freezing in liquid nitrogen and grinding. Washing salt from the tissue by dipping in molecular grade water may be helpful prior to DNA isolation, especially if enzymatic reactions are needed downstream (e.g., RADseq) as RNAlater contains EDTA which inhibits enzymatic activity.

If you are pre-filling tubes with RNAlater, do not overfill them (e.g., use 1.25 mL RNAlater in a 2 mL tube). You need enough solution to permeate the tissue well, as above, but remember that the tissue will displace volume, so pre-fill accordingly to avoid leakage.

Ingredients per 1000 mL liquid in solution (sourced at appropriate concentration, or made in lab):

Ingredient	Amount	Chemical formula	Molar mass	Preparation / Notes
0.5 M EDTA	40 mL	$C_{10}H_{16}N_2O_8$	372.24 g/mol	18.6 g / 100 mL ddH_2O, adjust pH to 8.0 with NaOH while stirring
1 M Sodium Citrate	25 mL	$Na_3C_6H_5O_7$	294.10 g/mol	29.4 g/ 100 mL ddH_2O, stir to dissolve
Ammonium Sulfate	700 g	$(NH_4)_2SO_4$		Displaces volume beyond 1000 mL of liquid, so leave plenty of space in flask.
Molecular grade water	935 mL	H_2O		
1 M Sulfuric acid	A few mL	H_2SO_4	98.08 g/mol	Use to adjust pH of the RNAlater solution to pH = 5.2

Equipment and materials needed:

- ☐ Autoclaved Erlenmeyer volumetric flask large enough to contain total volume with a good bit of excess space
- ☐ Autoclaved graduated cylinders
- ☐ Autoclaved beakers
- ☐ Autoclaved bottles with screw cap
- ☐ Magnetic stirrer bar, washed, sprayed with RNAse Away and dried
- ☐ Hot plate stirrer device
- ☐ Scale that can weigh hundreds of grams
- ☐ Aluminum foil
- ☐ Scoop
- ☐ pH meter
- ☐ pH meter calibration aliquots
- ☐ ddH_2O in a squirt bottle to clean pH meter probe
- ☐ 15 mL falcon tubes for pH test aliquots
- ☐ Plastic dropper pipettes
- ☐ Biohazard waste disposal receptacle
- ☐ 0.2 µm filtration units
- ☐ Hose for vacuum outlet

Make the RNAlater solution

1. Put magnetic stirrer bar in an Erlenmeyer volumetric flask with a flat bottom.
2. Combine 40 mL 0.5 M EDTA, 25 mL 1M Sodium Citrate, and 935 mL molecular grade water in the flask. Cover the mouth of the flask with foil to prevent things falling into it or dust getting in.
3. Place flask on hot plate stirrer plate, turn the temperature to 30°C, and the stirring speed to 300-400. Make sure that the liquid is stirring smoothly.
4. Place a beaker on the scale, and weigh out 100 g Ammonium Sulfate. Slowly pour the salt into the flask.
5. Repeat the measuring and adding of salt until you have added 700 g Ammonium Sulfate to the flask.
6. Adjust the temperature to 100°C and increase the speed of stirring enough that the liquid and salt are moving around, but not so much that the magnetic stirrer bar flies off center. Make sure the solution is being stirred.
7. Leave to stir for an hour (or more) until all the salt is dissolved.
8. Turn off the heat and leave to stir for an hour (or more) to fully incorporate salt and to reach room temp.

Adjust pH

Use the pH meter in the lab to measure the pH. You will need to adjust the pH to 5.2 by adding drops of sulfuric acid. The pH can change quickly, so work slowly because you don't want to overshoot the 5.2 pH goal.

1. Make sure that the pH meter glass bulb is submerged in the electrode solution (found next to the pH meter device). If not already present, prepare aliquots of the calibrating solutions: solution 1 has pH 4; solution 2 has pH 7; solution 3 has pH 10.
2. Turn on the pH meter device and rinse the pH meter bulb by squirting it with ddH2O over an empty beaker. Shake off excess water.
3. Hit the "calibrate" button, and place the pH meter in solution 1; wait until it shows a stable pH – ideally pH 4 – and the screen says "ready". Hit "OK" and repeat for solution 2 (pH 7) and solution 3 (pH 10).
4. Once the solution is no longer warm (room temperature is good), aliquot 3-5 mL in a 15 mL conical tube for baseline pH testing.
5. Test the pH by submerging the pH meter glass bulb in the RNAlater solution; put the aliquot tube in a rack rather than holding it in your hand to keep it still while the pH is being measured.
6. Typically, the RNAlater will be more basic than desired (that is, the pH will be higher than 5.2). Depending on your starting pH, work to reduce the pH using sulfuric acid. For example, if you start with pH 6.8, add 10 drops of sulfuric acid while the solution is stirring on the stirrer plate (heat off), wait for the acid to be incorporated, and then pour out a new aliquot to measure the new pH.
7. Measure the pH of the new aliquot as before: wash off the pH meter, shake off excess water, submerge pH meter bulb in solution, and wait until the screen displays a stable pH and says "ready."
8. Repeat this procedure to get closer to the target pH. As you get closer, add fewer drops of sulfuric acid at a time to avoid overshooting the target. Remember that the pH scale is logarithmic, so a few drops can make a big difference in pH. Continue until you reach pH = 5.2.

Filter the solution to sterilize

1. Obtain a filter unit; if it comes as just the top part with the filter, screw it onto the top of an autoclaved glass bottle. If it comes as the top and bottom together, make sure that the two parts are screwed together tightly.
2. Affix the hose onto the vacuum outlet on the lab bench, turn on the vacuum, and make sure it's working.
3. Open the lid of the filter unit attached to the bottle, pour in RNAlater solution, and close the lid.
4. With the vacuum turned on, attach the vacuum hose to the filter unit, and make sure the lid of the unit is firmly closed.
5. Watch to make sure that the liquid is filtering down through the filter unit.
6. Once all the liquid has filtered down, turn off the vacuum, remove the filter top, and cap the bottle.
7. Label the bottle with the contents, date, and your name.

RNAlater solution can be stored at room temperature. Mix well by swirling before dispensing into tubes.