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Tissue Collection and Storage in RNAlater

RNA later has been extensively used to store animal tissues, including brain, heart, kidney, spleen, liver, testis, muscle, fat, lung, and thymus. It penetrates into most biological tissues, and immediately stabilizes RNA (and DNA) in fresh specimens.

Recipe:

Materials:

- 40 mL 0.5 M EDTA disodium dehydrate (18.61 g/100 mL, bring the pH to 8.0 with NaOH while stirring – this usually takes 18-20 g of NaOH which should be added slowly while constantly monitoring the pH).
- 25 mL 1 M sodium citrate (trisodium salt dehydrate 29.4 g/100 mL, stir to dissolve)
- 700 g ammonium sulfate (powdered)
- ~ 900-950 mL sterile water
- 1 M H_2SO_4

Method:

- In a 1000 mL or 1500 mL beaker, combine 40 mL 0.5 M EDTA, 25 mL 1 M sodium citrate, and 700 g ammonium sulfate.
- Add sterile water and bring the combined volume to 1 L. Put the beaker on a hot plate and stir the solution with a magnetic stirrer on a low heat until ammonium sulfate is completely dissolved.
- Allow the mixture to cool, adjust the pH of the solution to 5.2 using 1M H_2SO_4
- Filter the solution via vacuum filtration in two stages to remove impurities. Use 1.2 micron glass fiber filter in the first round of filtration, followed by 0.8 or 0.45 micron glass fiber filter for the second round of filtration.
- Transfer the solution to a screw cap bottle and store either at room temperature or at 4°C.

Final concentrations:

- 20 mM EDTA, 25 mM sodium citrate, 70 g ammonia sulfate/100mL, pH 5.2

Important notes regarding use of RNAlater:

- Large tissues should be cut into smaller pieces (approximately 0.5-1 cm^3).
- Once the tissue is prepared, place it in 8-10x volumes of RNAlater. Having more RNAlater is always a good idea to ensure whole tissue or all tissue chunks are well covered with the solution.
- Keep the tissue in RNAlater for at least 8-10 hours (or overnight) at room temperature to ensure solution has penetrated into the tissue. After this, transfer it to -20°C or -80°C for long-term storage.

Note: Most tissue samples can be kept in RNAlater solution for 1 week at room temperature, up to 1 month at 4°C, or indefinitely at -20°C or -80°C.

- When you are ready to extract RNA, take the tissue out of RNAlater solution and dab it on a kimwipe to remove excess solution from the tissue sample before proceeding with the digestion step.
  Optional: If the tissue is intact, it is also ideal to wash it with deionized water to remove excess RNAlater solution before dabbing it on a kimwipe.