

Visualization of RNA Preparations on 1% Agarose Gels
AfCS Procedure Protocol ID PP0000002500
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This method is used to assess (roughly) the integrity of total RNA samples by visualization of discrete 18S and 28S ribosomal RNAs. Total RNA is separated by electrophoresis through a 1% agarose gel containing 1.3 μ M ethidium bromide. Binding of the ethidium bromide to the RNA allows visualization of the separated RNA molecules when the gel is exposed to ultraviolet (UV) light.

Preparation of the Agarose Gel

1. Combine the following in a glass container (200-ml bottle or 250-ml Erlenmeyer flask): 90 ml purified water; 1 g agarose; 10 ml 10X Tris-borate electrophoresis buffer (10X TBE).
2. Cap the bottle or cover the flask but do not seal.
3. Microwave for 2 min on high power.
4. Swirl to mix the solution. Avoid boiling over.
5. Microwave at high power for an additional 3 min.
6. Use gloves to remove the bottle or flask from the microwave and mix thoroughly by swirling (all of the agarose should be dissolved). Let the solution cool at room temperature to allow handling with bare hands. Do not over-cool, allowing the solution to solidify.
7. Add 5 μ l of 25 mM ethidium bromide (EtBr) to the cooled 1% agarose solution.
8. Swirl to mix.
9. Place a 20-well separation comb (1.5-mm slots) into the tray of the horizontal minigel electrophoresis system. Pour approximately 80 ml of the warm agarose solution into the tray.
10. Allow the agarose to solidify for at least 45 min at room temperature.

Separation of RNA on the Agarose Gel

11. Prepare RNA samples by mixing 1 μ g of total RNA with 2 μ l of 6X gel-loading dye and nuclease-free water to a total volume of 12 μ l.
12. Remove comb from the solidified gel.
13. Place the tray with gel into electrophoresis minigel box. Submerge the gel with 1X TBE (diluted from 10X TBE).
14. Apply 12 μ l of the 1 Kb plus DNA ladder (Kb ladder) to the first well by submerging the tip of the pipette into the top of the well and slowly dispensing contents.
15. Repeat step 14 for application of samples into subsequent wells.
16. Cover the minigel box (electrodes should be engaged). Samples will migrate towards the positive electrode.
17. Run gels at 100 V for 1.5 hr.
18. Stop the run, remove the gel tray, and visualize the image of RNA in the gel with the UV light box. Record the image with the camera.

Reagents and Materials

Agarose gel, 1%: ICN Biomedicals; catalog no. 820723

Tris-borate electrophoresis buffer, 10X (10X TBE): AfCS Protocol ID PS0000007900

Ethidium bromide (EtBr): AfCS Solution Protocol ID PS0000007600

Horizontal minigel electrophoresis system (Buffer Puffer self-recirculating system): Owl Separation Systems; catalog no. B3
Includes chamber; lid with power cords; UV gel tray; and 2 combs, 1.5 mm, 12- and 20-well

Gel-loading dye, 6X (6X loading dye): AfCS Solution Protocol ID PS0000007700

Nuclease-free water: Ambion; catalog no. 9930

Tris-borate electrophoresis buffer, 1X (1X TBE): AfCS Protocol ID PP0000008000

1 Kb plus DNA ladder (Kb ladder): AfCS Solution Protocol ID PS0000007800

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