

while handling RNA. Solutions should be made using autoclaved distilled water, or, whenever possible, autoclaved in suitable glass containers. (**Caution:** Toxic materials or volatile liquids should not be autoclaved.)

2. **Caution:** Fumes can be given off formaldehyde gels. Ideally they should be run in a fume hood, or at least run with a lid.
3. Formaldehyde gels do not stain well if ethidium bromide is added after electrophoresis is completed. If this is done, the bands are normally obscured by a general fogginess over the gel. However, this can be partially overcome if the gel is washed with 1% (w/v) glycine solution for 60 min prior to staining.
4. Many labs have reported that poor quality formamide is the most likely source of degradation of RNA. Our experience has shown that the one listed here is good quality. As a matter of routine, however, it is essential that the solution is deionized by stirring for approx 30 min with 40 g/L Amberlite monobed mixed resin (BDH, Poole, Dorset, UK, Amberlite IRN-150L, formerly MB1). The resin is removed by filtration through Whatman no. 1 filter paper. The efficacy of the resin can be monitored by observation of a drop in pH. If there is no fall to neutral pH within this time, it is likely that the resin is exhausted and should be replaced with a fresh batch.
5. Gloves should be worn at all times when handling ethidium bromide solutions. Also, these solutions should be decontaminated by mixing with activated charcoal after use. Filter solid and destroy by incineration. The filtrate can be discarded into the drains. Gels containing the dye should also be destroyed by incineration.
6. Ultraviolet radiation is dangerous to the eyes. Ensure that suitable eye protection is worn at all times when working with UV light.
7. Glyoxal-modified RNA does not stain with ethidium bromide. Thus, it is essential to reverse the modification by soaking the gel in 0.1M NaOH before staining.
8. The condition of the RNA after the labeling reaction can be monitored by electrophoresing a small portion of it on a formaldehyde-containing gel, as described in **Subheading 3.1**. After examination of the gel under UV light, it can be dried-down and autoradiographed to confirm that the ^{32}P label is associated with full-length RNA.
9. The design of a suitable paper electrophoresis tank can be found in Brownlee (5).
10. The remaining pCp-labeled RNA is suitable for use in a variety of direct RNA sequencing protocols, such as those described by Donis-Keller et al. (6) and Peattie (7).

References

1. Boedtke, H. (1971) Conformation independent molecular weight determinations of RNA by gel electrophoresis. *Biochim. Biophys. Acta* **240**, 448–453.
2. Lehrach, H., Diamond, D., Wozney, J. H., and Boedtke, H. (1977) RNA Molecular Weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry* **16**, 4743–4751.