

RNA releases nucleoside 3' monophosphates, resulting in the transfer of the  $^{32}\text{P}$  phosphate group to the nucleotide originally at the 3' end of the RNA. This nucleotide can then be identified by its electrophoretic mobility on paper, allowing the identity of the 3'-terminal nucleotide to be deduced. The 3'-labeled RNA can also be used for direct RNA sequence analysis.

## 2. Materials

Store all materials at room temperature unless indicated otherwise.

### 2.1. Electrophoresis of RNA Through Formaldehyde-Containing Agarose Gels

1. Low electroendosmosis (EEO) agarose type I (Sigma, St. Louis, MO).
2. 10X MOPS buffer: 0.2M 3-*N*-morpholinopropane-sulphonic acid (Sigma), 50 mM sodium acetate, 10 mM EDTA, adjusted to pH 7.0 with NaOH. Autoclave. (**Note:** The solution will appear yellow after autoclaving. This has no apparent detrimental effect.)
3. Formaldehyde, 37% (v/v) solution in dH<sub>2</sub>O. **Caution:** Formaldehyde solution and vapors are extremely toxic. The solution should be stored in a ventilated area or a fume hood but not in the same location as hydrochloric acid. It should be used in a fume hood, but not by a person under 18 yr old.
4. 1% (w/v) Glycine solution.
5. Formamide. Puriss, assay >99% (obtained from Fluka, Dorset, UK). **Caution:** Formamide is a teratogen and is toxic by inhalation or contact with the skin. Take extreme care. Formamide dissolves certain types of plastic and can pass through disposable gloves. Should not be used by persons under 18. Store at  $-20^{\circ}\text{C}$  (*see Note 4*).

### 2.2. Glyoxal Treatment of RNA and Electrophoresis Through Tris-Acetate Agarose Gels

1. 30% (w/v) Glyoxal solution. **Caution:** Glyoxal is an irritant to the skin, eyes, and respiratory system.
2. 10X TEAc buffer: 0.4M Tris base, 0.2M sodium acetate, 20 mM EDTA. Adjust pH to 7.5 with glacial acetic acid. Autoclave before use.
3. 10% (w/v) Sodium dodecyl sulfate (SDS) solution.
4. GFP solution: 80% (v/v) deionized formamide, 0.75M deionized glyoxal in 10 mM sodium phosphate, pH 7.0. Make small volumes and dispense into 100- $\mu\text{L}$  aliquots. Store at  $-70^{\circ}\text{C}$ .
5. 5X Loading dye: 20% (w/v) Ficoll 400, 1% (w/v) orange G, 5 mM EDTA, pH 7.0. Autoclave. Store aliquots frozen at  $-20^{\circ}\text{C}$ .
6. 0.1M NaOH.
7. 0.15M Sodium acetate (NaOAc), pH 5.5.
8. Ethidium bromide solution: Stock: 10 mg/mL in dH<sub>2</sub>O. **Caution:** Extreme care. Ethidium bromide is a powerful mutagen and is moderately toxic. Take appropriate measures to dispose of solid waste correctly (*see Note 5*). Store at  $4^{\circ}\text{C}$ .