

## Native Acrylamide Gel Electrophoresis

### Constant Temperature Gel w/o Mg<sup>+2</sup> in the Running Buffer

<b>1x Sample Loading Buffer:</b>	<b>10 x Stock Loading Dye</b>
½ x TBE	
1.5 % Glycerol	15 % Glycerol
0.01% Bromophenol Blue	0.1% Bromophenol Blue

(To make up a sample use e.g. 5 µl RNA/DNA sample, 4 µl 1 x TBE, and 1 µl 10 x Loading Dye). Final RNA load concentration: ~ 10-20 µM.

### Running Buffer

½ x TBE

### Gel Matrix

15% Acrylamide (75:1 Acryl:Bis)

½ x TBE

(Polymerize w/ Ammonium persulfate (APS) and TEMED – typically using 100λ 10% APS Stock and 10λ TEMED per 10mls of gel.)

### 15% Acrylamide (75 : 1 Acrylamide : Bisacrylamide; ½ x TBE) (1 Liter)

148 g Acrylamide  
2 g Bisacrylamide  
50 ml 10 x TBE

### ½ x TBE (1 Liter)

5.45 g TRIS Base  
2.80 g Boric Acid  
2.0 ml 0.5M EDTA (pH 8.0)

### 10x TBE (1 Liter)

109 g TRIS Base  
56 g Boric Acid  
40 ml 0.5M EDTA (pH 8.0)

### Constant Temperature Gel w Mg<sup>+2</sup> in the Running Buffer

<b>1x Sample Loading Buffer:</b>	<b>10 x Stock Loading Dye</b>
1 x TBM	
1.5 % Glycerol	15 % Glycerol
0.01% Bromophenol Blue	0.1% Bromophenol Blue

### Running Buffer

1 x TBM

### Gel Matrix

15% Acrylamide (75:1 Acryl:Bis)

1 x TBM

(polymerize w/ APS and TEMED)

**15% Acrylamide (75 : 1 Acrylamide : Bisacrylamide; 1 x TBM) (1 Liter)**

148 g Acrylamide

2 g Bisacrylamide

100 ml 10 x TBM

**1 x TBM (1 Liter)**

10.9 g TRIS Base

5.6 g Boric Acid

2.0ml 0.5M MgCl<sub>2</sub>

**10x TBM (1 Liter)**

109 g TRIS Base

56 g Boric Acid

20 ml 0.5M MgCl<sub>2</sub>

Gels are run at 4 °C using a constant temperature submarine Minigel Apparatus from BioRad. (using ~ 10V/cm of gel)

Bands on the gel are stained using ~ 0.5 µg/ml ethidium bromide solution (EtBr) and viewed using a UV illuminator.

**CAUTION:** EtBr is a powerful mutagen and is moderately toxic – always wear gloves when working with EtBr!

EtBr solutions should be decontaminated before discarding them.