

Buffers For RNA Gel

1% Agarose Formaldehyde gel : 100mls \Rightarrow 1g agarose

10X MOPS - 10mls - $\frac{150\text{mls}}{15\text{mls}}$ * Microwave agarose in H₂O to dissolve. Cool to 60°C then add MOPS and HCHO.

HCHO 37% - 5.4mls - 8.1mls

DEPC H₂O - $\frac{84.6\text{mls}}{100\text{mls}}$ - $\frac{126.9\text{mls}}{150\text{mls}}$

Running Buffer : 1 liter

10X MOPS - 100mls

* Use ddH₂O but first run tap a few secs before collecting H₂O.

HCHO 37% - 53.6mls

DEPC H₂O - $\frac{846.4\text{mls}}{1000\text{mls}}$

Formaldehyde Gel-Loading Buffer : 50mls

10X MOPS - 5mls

BPS - 0.25%

HCHO 37% - 8.9mls

XC - 0.25%

Glycerol - 25mls

EtBr - 12 μ g/ml (100 μ g)

0.5M EDTA - 100 μ l

DEPC H₂O - $\frac{11.1\text{mls}}{50\text{mls}}$

10X MOPS Buffer : - 1 liter

400mM MOPS Acid - 83.7g

* Filter Sterilize thru' 0.2 μ nitrocellulose filters.

100mM NaH₂CO₃ - 13.6g

20mM EDTA - 40mls (0.5M)

DEPC H₂O - $\frac{\text{to } 1000\text{mls}}{1000\text{mls}}$

Sample Preparation : 20 μ l

RNA	- 4.5 μ l	* add 2.0 μ l loading dye
10x MOPS	- 2.0 μ l	just prior to loading.
HCHO 37%	- 3.5 μ l	
HCHO/NH ₂	- 10.0 μ l	
	<u>20.0 μl</u>	

Electrophoretic Conditions

- Soak Gel apparatus in dilute bleach or absolute detergent for a few hours
- Rinse thoroughly in RO H₂O
- Dry gel rig and place gel in tray into apparatus in chemical hood.
- load gel and run at 30 volts o/n. Not necessary to recirculate buffer.
- Prior to loading gel. Heat samples 65°C 5-10 mins then place on ice immediately.
- Centrifuge to get sample to bottom of eppendorf.
- load gel