

# Certificate of Analysis

## RNA Markers:

Part No.	Size
G319A	50µl

**Description:** Promega's RNA Markers<sup>(a)</sup> are suitable for size estimation of single-stranded RNA from 0.28–6.58kb in glyoxal or formaldehyde agarose gels. The RNA Markers consist of a ladder of nine RNA transcripts that are synthesized in vitro from specific templates. The sizes are 281, 623, 955, 1,383, 1,908, 2,604, 3,638, 4,981 and 6,583 bases. Three microliters of RNA prepared in formaldehyde/MOPS Buffer are separated onto a 1% formaldehyde-agarose gel using MOPS running buffer. After electrophoresis, bands are visualized by ethidium bromide staining.

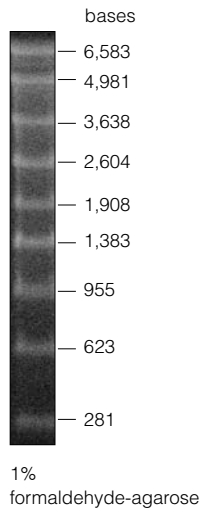
**Note:** We do not recommend use of these markers as a quantitative standard.

**Storage Buffer:** RNA Markers are supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

**Storage Conditions:** Store at –70°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes by storing in single-use aliquots. See the expiration date on the Product Information Label.

**Usage Notes:** To optimize performance of the RNA Markers, we recommend the following procedures for use in all applications.

1. Avoid RNase contamination of both samples and RNA Markers. Use sterile, disposable plasticware whenever possible.
2. Thaw the RNA Markers on ice.
3. After thawing, spin the tube containing the markers briefly in a microcentrifuge to collect any material that may be present around the rim of the tube. **Mix the markers extremely well before use** by pipetting and vortexing, as concentration gradients may form in frozen products and should be dispersed upon thawing.
4. Load 3µl of the RNA Markers per lane on agarose gels. Use of these markers on acrylamide gels is not recommended.
5. Treat the RNA Markers in the same manner as your RNA samples.



Part# 9PIG319

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**Promega**

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Signed by:

R. Wheeler, Quality Assurance

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## I. Gel Preparation and RNA Visualization

Two protocols for gel preparation are provided: denaturing (MOPS) and nondenaturing (TAE buffer). References 1 and 2 contain additional protocols for running RNA samples on denaturing gels.

**Note:** Although denaturing gels containing glyoxal, formaldehyde or 8M urea provide the greatest resolution of denatured RNA, acceptable results can be obtained using nondenaturing gels and RNA denatured in a formaldehyde/formamide sample buffer.

### A. MOPS Buffer Protocol

1. Prepare a 1% agarose/formaldehyde gel:

20.0ml	5X MOPS buffer
62.0ml	autoclaved NANOpure® water
1.0g	agarose, molecular biology grade
2. Heat to boiling (in a microwave oven) and cool to  $-55^{\circ}\text{C}$ . Move reagents and materials to a fume hood, add 17.6ml 37% formaldehyde to the mixture and gently stir.

**Caution:** Formaldehyde vapors are toxic.

3. Cast gel using a comb with  $4 \times 2\text{mm}$  teeth for best results.
4. Add  $3\mu\text{l}$  of RNA Markers to  $18\text{--}20\mu\text{l}$  of RNA sample buffer.
5. Add  $2\text{--}5\mu\text{l}$  of RNA loading buffer and heat the sample for 10 minutes at  $65^{\circ}\text{C}$ , and immediately place on ice for 2 minutes prior to loading.
6. Run gel at 60V for approximately 90 minutes or until bromophenol blue dye front has migrated about 6cm down the gel (from wells).

### B. TAE Buffer Protocol

1. Prepare an agarose gel in 1X TAE.
2. Add  $3\mu\text{l}$  of RNA Markers to  $18\text{--}20\mu\text{l}$  of RNA sample buffer.
3. Add  $2\text{--}5\mu\text{l}$  of RNA loading buffer and heat the sample for  $5\text{--}10$  minutes at  $65\text{--}70^{\circ}\text{C}$  prior to loading.
4. Run the gel under standard conditions for the analysis of nucleic acid samples.

## II. Composition of Buffers and Solutions

### RNA sample buffer

10.0ml	deionized formamide
3.5ml	37% formaldehyde
2.0ml	5X MOPS buffer

Dispense into single-use aliquots in tightly sealed screw-cap tubes and store at  $-20^{\circ}\text{C}$ . These aliquots can be stored for 3–6 months. Do not freeze-thaw.

### RNA loading buffer

50%	glycerol
1mM	EDTA
0.4%	bromophenol blue
1mg/ml	ethidium bromide

Make up to 20ml using a very high grade glycerol (e.g., Fisher Cat.# G153-4). The RNA loading buffer should be dispensed into single-use aliquots and stored at  $-20^{\circ}\text{C}$ .

### TAE 50X buffer (pH 8.3)

242g	Tris base
57.1ml	glacial acetic acid
100ml	0.5M EDTA stock (pH 8.0)

Add the Tris base and EDTA stock to 500ml of deionized water. Add the glacial acetic acid. Bring to 1 liter with deionized water.

### 5X MOPS buffer

0.2M	MOPS (pH 7.0)
50mM	sodium acetate
5mM	EDTA (from a stock of 0.5M EDTA [pH 8])

## III. References

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. Ausubel, F.M. *et al.* (1993) *Current Protocols in Molecular Biology*, Vol. 2, Greene Publishing Associates, Inc., and John Wiley and Sons, NY.