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# Reliant® RNA Gel System

**For agarose gel RNA electrophoresis**

## Introduction

Reliant® RNA Gel System precast agarose gels are formulated for separation of RNA of sizes from 0.25 kb to at least 10 kb. The gels consist of 1.25% SeaKem® Gold Agarose in 1X AccuGENE® MOPS Buffer with no denaturants and are stable for 1 year at room temperature. They are free of contaminating RNases, thus saving time and effort preparing RNase-free agarose gels. The gel is self-contained in a plastic tray which eliminates the need to decontaminate your electrophoretic equipment to ensure it is RNase free.

In most cases it is possible to run RNA in a denaturing sample buffer on native agarose gels and maintain the RNA molecules in a denatured state. The absence of denaturants in the gel eliminates problems associated with toxic chemicals and the background problems associated with staining gels containing formaldehyde. To denature RNA, use one of the RNA sample denaturing procedures suggested in this protocol.

### Suggested Applications

- Analysis of *in-vitro* synthesized transcripts
- Checking integrity of whole-cell RNA
- Fractionating and recovering RNA

## Detection Limits

RNA that is not glyoxalated and stained with GelStar® Nucleic Acid Gel Stain can be detected at a level of 1 ng/band. Glyoxalated RNA can be detected at 100ng/band when stained with GelStar® Stain. Ethidium bromide-stained RNA can be detected at quantities 10 to 20 times higher.

## Precautions

Formamide, formaldehyde, glyoxal and DMSO are hazardous materials. Gloves, lab coat and safety glasses should be worn when handling these materials, and other appropriate protective measures should be used to avoid exposure, refer to MSDS for more detail.

Use standard methods to protect your samples from RNase contamination. For more information on this and other associated techniques see:

Ausubel, F.M., *et al.* (eds.) *Current Protocols in Molecular Biology*, John Wiley & Sons, 1988.
Sambrook, J., *et al*. *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Press, 1989.

## Running Buffer

Prepare 10X MOPS running buffer with the following components, or use Lonza's AccuGENE® 10X MOPS Buffer.

| | g/L |
|---|---|
| 200 mM MOPS (free acid) | 41.86 g |
| 50 mM sodium acetate | 6.80 g |
| 10 mM EDTA•2$H_2O$ | 3.72 g |
| 10 mM EGTA (free acid) | 3.80 g |

Use RNase-free chemicals, water and containers.
Mix with 850 ml water.
Adjust pH to 7 with 10 M NaOH.
Adjust final volume to 1 liter.
Filter through a 0.2 μm nitrocellulose filter and store in the dark.

## Gel Loading Buffer

50% glycerol
1 mM EDTA
0.6% bromophenol blue
0.6% xylene cyanol

## Sample Preparation

### No Denaturation

1. Bring RNA up to a volume of 8 μl with RNase-free water or 1X MOPS buffer.

### Formamide-Only Denaturation

1. Bring RNA up to a volume of 8 μl with RNase-free water.
2. Add:
   2 μl 10X MOPS
   9 μl deionized formamide
3. Mix and heat samples for 10 min. at 70°C, then chill on ice for at least one minute.

### Formaldehyde Denaturation

1. Bring RNA up to a volume of 6 μl with RNase-free water.
2. Add:
   2 μl 10X MOPS
   2 μl 37% formaldehyde
   9 μl deionized formamide
3. Mix and heat samples for 10 min. at 70°C, then chill on ice for at least one minute.

### Glyoxal Denaturation

1. Prepare Glyoxal/DMSO mixture
   - Mix 2.5 ml DMSO with 1.5 ml deionized glyoxal and 1 ml 10X MOPS buffer.
   - Quickly dispense small aliquots into microcentrifuge tubes and store at -20°C.
   - Thaw each aliquot only once – **do not reuse.**
2. Bring RNA up to a volume of 9 μl with RNase-free water.
3. Add: 9 μl Glyoxal/DMSO mixture.
4. Mix and heat samples for 60 min. at 50°C, then chill on ice for at least one minute.

## Electrophoresis

1. Dilute the 10X MOPS buffer to 1X with RNase-free water.
2. Peel the cover from the Reliant® Gel tray.
3. Remove the protective strips from the tape on the bottom of the tray, and press the tray onto the electrophoresis chamber platform.
4. Pour the 1X MOPS buffer into the electrophoresis chamber, covering the gel tray flange to a depth of 5 mm.
5. Add 1 µl of gel loading buffer to each sample and mix.
6. Load up to 20 µl of RNA sample to gel.
7. Electrophorese for 2 hours at a field strength of 3.5 V/cm (cm = interelectrode distance).

## Detection of RNA

**To stain non-glyoxalated RNA with GelStar® Nucleic Acid Gel Stain or SYBR® Green II Gel Stain**

1. Dilute the stain 1:10,000 in TE buffer.
2. Remove the gel from the tray and transfer to a clean polypropylene container.
3. Cover the gel with the diluted stain, then shake gently for 30 minutes.
4. Detect the RNA using standard 300 nm UV transillumination or other photo-documentation device.

**To stain glyoxalated RNA with GelStar® Stain or SYBR® Green II Gel Stain**

1. Dilute the stain 1:5,000 in 100 mM ammonium acetate.
2. Follow steps 2-4 in the protocol for staining non-glyoxylated RNA.

**NOTE: For details on GelStar® Stain or SYBR® Green Stain use, refer to the individual product protocols.**

**To stain non-glyoxalated RNA with ethidium bromide:**

1. Dilute stock ethidium bromide to 0.5 µg/ml in water.
2. Remove the gel from the tray and transfer to a clean polypropylene container.
3. Cover the gel with the diluted ethidium bromide solution, then shake gently for 30 minutes.
4. Rinse the gel with water, then destain for 30 minutes in water.
5. Visualize using standard techniques.

**To stain glyoxalated RNA with ethidium bromide:**
**NOTE: Ethidium bromide is the least sensitive stain especially when used to stain glyoxalated RNA.**

1. Dilute stock ethidium bromide to 0.5 µg/ml in 100 mM ammonium acetate.
2. Remove the gel from the tray and transfer to a clean polypropylene container.
3. Cover the gel with the diluted ethidium bromide solution, then shake gently for 30 minutes.
4. Rinse the gel with water, then destain for 30 minutes in 100 mM ammonium acetate.
5. Visualize using standard techniques.

## Ordering Information

| Catalog No. | Description | Size |
|---|---|---|
| 54922 | Reliant® RNA Gel System 8 well | 20 Gels |
| 54948 | Reliant® RNA Gel System 20 well | 20 Gels |
| 50561 | Reliant® RNA Gel System 8 well with glyoxal sample buffer 1 x 1.7 ml glyoxal buffer | 20 Gels |

## Related Products

GelStar® Nucleic Acid Gel Stain
SYBR® Green II Nucleic Acid Gel Stain
AccuGENE® 10X MOPS Buffer
Reusable UV Transparent Tray Landscape tray for the Reliant® Gel System
Reusable UV Transparent Tray Portrait tray
RNA Markers 0.5-9 kb
Glyoxal Sample Buffer

**For more information contace Technical Service at (800) 521-0390 or visit our website at www.Lonza.com.**

**For Laboratory Use.**