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ProductInformation

PRECAST AGAROSE GELS
For RNA Electrophoresis

Product No. **P 6222**

Product Description

Sigma's Precast Agarose Gels for RNA Electrophoresis are suitable for separating RNA sizes from 0.25 to 10 kb. The 8-well gels are 1.25% RNase-free agarose (Product No. A 9539) prepared using 1X MOPS buffer with no denaturants. The gels are formulated without formaldehyde and are recommended for the analysis of total RNA, *in vitro* RNA transcripts and Northern blotting.

Denaturation of the RNA is usually not necessary before loading the gel. However, if significant secondary structure of the RNA is suspected, the RNA should be denatured using one of the protocols included in the procedure section.

Precautions and Disclaimer

Sigma's Precast Agarose Gels for RNA electrophoresis are for laboratory use only. Not for drug, household or other uses. Many reagents used in electrophoresis are hazardous. Warning statements are included on the label. Always wear personal protective equipment including gloves and eye protection when handling. Refer to Material Safety Data Sheet.

Storage

Store flat at room temperature (18 °C-25 °C). See product label for expiration date.
Do Not Freeze.

Product Profile

Sigma's Precast agarose gels for RNA analysis are suitable for use with Bio-Rad Mini-Sub®, Hoefer Minnie the Gel-Cicle® , Life Technologies Horizon® 58 and Sigma submarine mini-gel (Product No. E 0638) electrophoresis units.
Gel Descriptions
Tray dimension: 6.8 cm x 10.2 cm
Gel dimension: 6.0 cm x 9.5 cm
Gel thickness: 5.5 mm
Sample format: Gels are cast with 8 wells that will each accommodate up to 15 µl sample volume.

Reagents Required but Not Provided

- 10X MOPS running buffer, Product No. M 5755 (0.4 M MOPS, pH approx. 7.0, 0.1 M sodium acetate, 10 mM EDTA)
- RNA sample loading buffer, Product No. R 4268 (with 50 µg/ml ethidium bromide) or Product No. R 1386 (without ethidium bromide); 62.5% (v/v) deionized formamide, 1.14 M formaldehyde, 200 µg/ml bromophenol blue, 200 µg/ml xylene cyanole, 1.25 X MOPS-EDTA-sodium acetate, **or**
- Gel loading solution, Product No. G 7654 (40% (w/v) sucrose, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanole FF)

Additional reagents required for specific denaturation methods:
Formamide, Product No. F 7508
Dimethyl sulfoxide, Product No. D 8418
Glyoxal, Product No. G 3140
Mixed Bed Resin (for deionization of formamide or glyoxal), Product No. M 8032. Deionization procedure is included with the resin.

Procedure

Prepare sample using one of the following procedures:

A. No denaturation required
Bring RNA up to a volume of 8 µl with RNase-free water or 1X MOPS buffer. Add 2 µl of gel loading solution (Product No. G 7654).

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

C. Formaldehyde denaturation
Bring RNA up to a volume of 6 µl with RNase-free water. Add 12 µl RNA sample loading buffer (with or without ethidium bromide). Mix and heat samples for 10 minutes at 70 °C, then chill on ice for at least one minute.

D. Glyoxal denaturation
Prepare glyoxal/DMSO mixture by mixing 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 –80 °C. Thaw each aliquot only once; do not reuse.

Bring RNA up to a volume of 9 µl with RNase-free water. Add 9 µl glyoxal/DMSO mixture. Mix and heat samples for 60 minutes at 50 °C, then chill on ice for at least one minute.

Sigma's Precast agarose gels for RNA analysis require less than 5 minutes to set up.

1. Peel the paper backing from the adhesive strips on the bottom of the tray.

2. Peel off the lid. **Leave the gel in the tray.**

3. Press the tray directly onto the chamber platform. Align the wells so the RNA samples will run straight.

4. Pour 1X MOPS running buffer in the chamber to a **depth of 5 mm** OVER the flange of the tray.

5. Load the RNA sample (≤15 µl volume).

6. Electrophorese the gels at 3.5 V/cm for 2 hours. Lower voltages for longer times are acceptable.

7. Remove the gel from the tray to photograph/document and/or destain.

Reference

RNA Methodologies: A Laboratory Guide for Isolation and Characterization, R.E. Farrell Jr., Academic Press Inc., San Diego, CA, 1993 (Product No. Z35,035-4)

A Laboratory Guide to RNA: Isolation, Analysis and Synthesis, P. Krieg, Ed., Wiley-Liss, New York, NY, 1996 (Product No. Z37,382-6)

Short Protocols in Molecular Biology, 3rd ed., F.M. Ausubel, *et al*., John Wiley & Sons, Inc., New York, NY, 1995 (Product No. Z36,436-3)

Molecular Cloning: A Laboratory Manual, 2nd ed., Vol. 1, 2, and 3, J. Sambrook, E.F. Fritsch and T. Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989 (Product No. M3401)

Related Products

RNaseZAP, Product No. R 2020
RNA markers:
0.28-6.6 kb, Product No. R 7644
0.36-9.5 kb, Product No. R 3137
RNA marker template set, Product No. R 4142

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