Denaturing formaldehyde agarose gel-electrophoresis

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Abstract

Protocol for separating total RNA using denaturing formaldehyde agarose gel electrophoresis.

This method can be used to separate larger RNAs in a range of 400-6000 nt, either for quality control or downstream Northern Blot Analysis.

Citation: Anna Behle Denaturing formaldehyde agarose gel-electrophoresis. **protocols.io** dx.doi.org/10.17504/protocols.io.gtsbwne
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Guidelines

Always wear gloves when handling hazardous materials.

Always handle RNA cold and in an RNase free manner.

Protocol

Buffers and reagents needed:

Step 1.

- 10x MEN buffer: 200 mM MOPS, 50 mM NaOAc, 10 mM EDTA, pH = 7.0
- Formaldehyde
- Ultra pure water
- Agarose

Gel preparation

Step 2.

Recipe for one 100 mL gel (e.g. PeqLab Mini L electrophoresis chamber):

Assemble gel chamber with suitable comb.

Heat 1.3 g in 85 mL ultra pure water. Let cool.

Under the hood, quickly add 10 mL 10x MEN buffer and 5 mL formaldehyde and pour gel.

RNA preparation

Step 3.

For Northern Blot Analysis, 1-3 µg of RNA should be loaded per well.

Adjust concentration and volume of all RNA samples by adding RNase-free ddH_2O. Add equal amounts of 2x RNA Loading Dye.

Denature RNA at 95˚C for 5 min. Snap cool on ice.

RNA loading and electrophoresis

Step 4.

Add 1x MEN to the chamber (running buffer). Make sure wells are covered in buffer.

Load RNA.

Separate RNA at 5-6 V/cm for at least 30 minutes or longer, if desi

Visualization

Step 5.

Document the gel using the BioRad ChemiDoc MP Imaging System.

Use either the GelRed or the Ethidium bromide protocol.

Warnings

Wear gloves when handling formaldehyde and ethidium bromide.

Keep formaldehyde and gel under fume hood.