Preparation of Denaturing Agarose Gel for RNA Analysis

David Dunigan and Irina Agarkova

Abstract

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Protocol

Gel Preparation

Step 1.
Melt 1.0 g agarose in 87 ml of DEPC water, by dispersing the agarose uniformly and heating in a microwave until all particles are dissolved.

Gel Preparation

Step 2.
Bring the melted agarose to 60 °C.

Gel Preparation

Step 3.
Add 10 ml 10X MOPS Buffer and 3 ml 37% formaldehyde.

PROTOCOL

. 10X MOPS Buffer

CONTACT: Irina Agarkova

NOTES

Irina Agarkova 31 Mar 2016
Use DEPC treated water and RNase free reagents.

Step 3.1.
10X MOPS consists of the following (per liter):

Step 3.2.
0.2 M MOPS pH 7 with NaOH

Step 3.3.
50 mM sodium acetate

Step 3.4.
10 mM EDTA

Gel Preparation

Step 4.
FORMALDEHYDE IS VOLATILE AND TOXIC. WORK IN A HOOD FROM THIS POINT FORWARD.

Gel Preparation

Step 5.

Pour standard agarose gel gel. USE A FUME HOOD!

Gel Preparation

Step 6.
Allow gel to set for 1 hour.

DURATION
01:00:00

Gel Preparation

Step 7.
Run gel in 1X MOPS Buffer.

PROTOCOL

. 10X MOPS Buffer

CONTACT: Irina Agarkova

Step 7.1.
10X MOPS consists of the following (per liter):

Step 7.2.
0.2 M MOPS pH 7 with NaOH

Step 7.3.
50 mM sodium acetate

Step 7.4.
10 mM EDTA

Sample Preparation

Step 8.
Mix 40 µl sample buffer with 10 µl sample, heat to 55 °C 15 minutes.

DURATION
00:15:00

NOTES
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The sample buffer is comprised of 65% formamide, 22% formalin (37% formaldehyde) and 13% 10X MOPS.

Sample Preparation

Step 9.
Add 10µl of the following:

- 50% glycerol
- 1 mM EDTA
- 0.3% each bromophenol blue and 0.3% xylene cyanol.

Warnings

Note: RNA is subject to rapid degradation by RNase present in the environment. For optimal results, use water treated with DEPC.