

Denaturing RNA Urea-PAGE

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Abstract

Protocol for separating total RNA under denaturing conditions using Urea-PAGE using the 10 mL Hoefer SE260 *Mighty Small* system.

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Before start

Thoroughly clean all components with ethanol and RNase away.

Heat block to 95°C.

Protocol

Gel preparation

Step 1.

Before preparing the gel, clean all components with 70 % ethanol and RNase away.

Recipe for one small Hoefer gel (10 mL):

PAA percentage	5 %	6 %	8 %	10 %	12 %	15 %
40 % PAA (19:1)	1.25 mL	1.5 mL	2 mL	2.5 mL	3 mL	3.74 mL
Urea	5 g	5 g	5 g	5 g	5 g	5 g
10x TBE	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
10 % APS	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL
TEMED	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL
ddH₂O	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL

Gel preparation

Step 2.

Dissolve 5 g urea in PAA, 10x TBE and H₂O. This can be done at RT or at 40°C. Agitate/shake

occasionally.

SAFETY INFORMATION

Wear vinyl gloves when handling PAA (neurotoxic!)

Gel preparation

Step 3.

Let the liquid cool down before pouring gel.

Assemble gel caster. Take a 1 mL aliquot of gel mixture, add 20 µL APS and 1 µL TEMED and quickly pour to prevent gel from running out.

Gel preparation

Step 4.

Add 80 µL APS and 12 µL TEMED to the rest of the gel and quickly pour. Insert comb immediately and clasp tight to prevent leakage. Let polymerize for an hour or over night.

Gels can be stored for a week by wrapping with wet paper towels and Saran wrap.

RNA preparation

Step 5.

For Northern Blot Analysis, at least 2-3 µg of RNA should be loaded per well.

Adjust concentration and volume of all RNA samples by adding RNase-free ddH₂O. Add desired amount of 2x or 5x RNA loading dye.

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

DURATION

00:05:00

SAFETY INFORMATION

RNA loading dye contains formamide. Wear goggles/lab coat/ gloves!

RNA loading

Step 6.

Add running buffer (1x TBE). Remove comb. Before loading samples, wash all wells and each well individually just before loading with 1xTBE and a syringe or a pipette tip. Carefully pipet samples into wells.

Step 7.

Close the lid and plug electrodes into power supply.


Separate RNA at 20 mA/gel, 1 hour.

Visualization

Step 8.

Pour out buffer, then disassemble gel.

Incubate gel in 0.5x TBE + GelRed for 10 minutes, then visualize.

 **DURATION**
00:10:00

Warnings

Wear vinyl gloves when handling PAA.

Make sure to thoroughly wash wells before loading samples.

2x RNA loading buffer contains formamide.