Mar 23, 2017

Denaturing RNA Urea-PAGE

DOI
dx.doi.org/10.17504/protocols.io.gszbwf6

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Protocol Citation: Anna Behle 2017. Denaturing RNA Urea-PAGE. **protocols.io https://dx.doi.org/10.17504/protocols.io.gszbwf6**

Protocol status: Working

Created: December 18, 2016

Last Modified: March 28, 2018

Protocol Integer ID: 4665

Abstract

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

Safety warnings

Wear vinyl gloves when handling PAA.
Make sure to thoroughly wash wells before loading samples.
2x RNA loading buffer contains formamide.

Before start

Thoroughly clean all components with ethanol and RNase away.
Heat block to 95˚C.

Gel preparation

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
	ddH_2O	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL

2 Dissolve 5 g urea in PAA, 10x TBE and H_2O. This can be done at RT or at 40˚C.
Agitate/shake occasionally.

Safety information

Wear vinyl gloves when handling PAA (neurotoxic!)

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.

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

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.
00:05:00

Safety information

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

RNA loading

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.

7 Close the lid and plug electrodes into power supply.
Separate RNA at 20 mA/gel, ~1 hour.

Visualization

8 Pour out buffer, then disassemble gel.
Incubate gel in 0.5x TBE + GelRed for 10 minutes, then visualize.
00:10:00