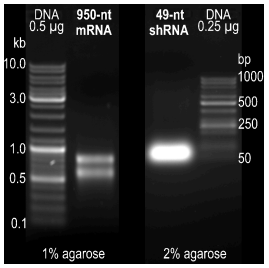
Sep 04, 2025

RNA agarose gel electrophoresis with bleach gels



In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.8epv5oymng1b/v1

Christian CR Renicke[1]

[1]Stanford University - Genetics

Christian CR Renicke
Stanford University - Genetics

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.8epv5oymng1b/v1

Protocol Citation: Christian CR Renicke 2025. RNA agarose gel electrophoresis with bleach gels. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5oymng1b/v1

Manuscript citation:
Renicke et al., 2025

Protocol status: Working
We use this protocol and it's working

Created: June 17, 2025

Last Modified: September 04, 2025

Protocol Integer ID: 220377

Keywords: rna agarose gel electrophoresis with bleach, rna agarose gel electrophoresi, gel electrophoresi, rna, transcription, bleach, gels in tae, added bleach, gel, agarose

Abstract

This protocol describes how to perform RNA non-denaturing gel electrophoresis in agarose-gels in TAE (or sodium borate) buffer with added bleach to assess their quality after routine *in vitro* transcriptions.

Image Attribution

Christian Renicke

Guidelines

All quantities and volumes in this protocol are for a 100-mL 1% agarose gel. This concentration works fine for RNA >500 nt, for shorter RNA, go up to 2.5% of agarose. Bands <500 nt will be increasingly diffuse. The TAE buffer can be substituted by a sodium borate buffer (for the gel and the electrophoresis chamber) which will yield sharper bands (see Materials section). Unfortunately, in this buffer, the bleach causes the gels to become rather fragile and kind of brittle, so they need to be handled very careful.

Although, the RNA is somewhat protected from RNases upon adding the loading dye, it is best to still keep it on ice and load it on the gel without much delay (the loading dye's slightly alkaline pH is not ideal for RNA stability but that is more on a longer time scale).

Materials

50× TAE electrophoresis buffer: **for 1 L:**

- 2 M Tris free base 242 g
- 0.05 M Disodium EDTA 18.61 g
- 1 M Glacial Acetic Acid 57.1 ml

Dissolve the Tris and EDTA in 700 mL of dH_2O under vigorous stirring. Add the acetic acid to the solution and fill the volume to 1 L with water. The pH should be around 8.2-8.5 and doesn't need adjustment. Sterilize by autoclaving or filtration (0.2 µm pore size) and store at room temperature.

1× TAE electrophoresis buffer:

- 40mM Tris
- 20mM Acetate
- 1mM EDTA

For 1 L, mix 20 mL 50× buffer stock with 980 mL dH_2O. The pH will slightly increase and should be around 8.6 (again, no adjustment needed). Store at room temperature.

Agarose, electrophoresis-grade

- Invitrogen™ UltraPure™ Agarose - 16500500

https://www.thermofisher.com/order/catalog/product/16500500

Bleach

- **CloroxPro Clorox Germicidal Bleach, Concentrated** - Mfr. Num. 30966

E.g., from **officedepot.com** or **grainger.com** (always confirm the Mfr. Num. before ordering and on receiving)

6× DNA gel loading dye:

- Thermo Scientific™ TriTrack DNA Loading Dye (6X) - R1161 (plenty supplied with ladders)

https://www.thermofisher.com/order/catalog/product/R1161

This dye contains 60% glycerol, 10 mM Tris-Cl (pH 7.6), 60 mM EDTA, 0.03% bromophenol blue, 0.03% xylene cyanol FF and 0.15% orange G.

DNA ladders:

- 100-10,000 bp: Thermo Scientific™ GeneRuler DNA Ladder Mix - SM0331

https://www.thermofisher.com/order/catalog/product/SM0331

- 50-1000 bp: Thermo Scientific™ GeneRuler 50-bp DNA Ladder - SM0371

https://www.thermofisher.com/order/catalog/product/SM0371

Before first usage, add 100 µL of the supplied TriTrack 6× DNA Loading dye and 400 µL nuclease-free water and mix. Store at room temperature. 6 µL of the resulting ready-to-use mix contain 0.5 µg DNA.

Nuclease-free water:

- *E.g.*, 2-mL aliquots from IDT - 11-04-02-01

https://www.idtdna.com/pages/products/reagents-and-kits/buffers-and-solutions

Ethidium bromide solution:

- 10 mg/mL in H_2O, e.g., Sigma-Aldrich - E1510

https://www.sigmaaldrich.com/US/en/product/sigma/e1510

OPTIONAL: For 1.5-3% agarose gels (works also in polyacrylamide gels):

20× sodium borate electrophoresis buffer:		**for 1 L:**
100 mM	sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; BORAX)	38.17 g
400 mM	boric acid ($H_3BO_{3)}$	25 g

Dissolve in 900 mL dH2O, adjust pH to 8.0 with boric acid (to decrease) or sodium hydroxide (to increase). Bring solution to 1 L with dH_2O. Sterilize by autoclaving or filtration (0.2 µm pore size) and store at room temperature.

1× sodium borate electrophoresis buffer:

- 5 mM sodium tetraborate
- 20 mM boric acid

For 1 L, mix 50 mL 20× buffer stock with 950 mL dH_2O. The pH will increase and should be around 8.5 (no adjustment needed). Store at room temperature.
2.5% agarose gels can be run at 10 V/cm or even higher and yield great separation and clear bands especially below 500 nt or bp.
One caveat is that the running behavior is sensitive to high salt or glycerol concentrations in the loaded sample, so glycerol-based 6× loading dye should not make up more than 1/6 of the sample volume or you might want to use Ficoll or sucrose-based loading dyes, salt should be no problem with the kit-purified RNA samples.
The other inconvenience is that adding bleach to sodium borate gels makes them very brittle and fragile.

Safety warnings

It is absolutely critical to use a bleach without added detergents, surfactants and chelating agents!

The **professional-grade** Chlorox**Pro** germicidal bleach has a higher hypochlorite content (8.25% vs. 5-6% for most household bleach products), the protocol works with both concentrations.
The retail bleach products from Chlorox all contain polydiallyldimethylammounium chloride, polyacrylic acid and/or other additives which interfere with the protocol (they most likely masking or changing the negative charge of the nucleic acids, in the worst case, the gels are blank). Only use bleach for which the full list of ingredients is available not just the "active ingredient", it should only contain the following:

- sodium hypochlorite
- sodium hydroxide
- sodium chloride
- sodium carbonate
- sodium chlorate

SAFETY REMARKS: Bleach is toxic and corrosive chemical. Handle with care, wear PPE when working with it. Be careful when handling hot liquids and be extra cautious when removing the agarose solution from the microwave, it might have been superheated and boil over upon agitation. Wear heat resistant gloves, eye protection and general PPE. Ethidium bromide is a suspected carcinogen, wear gloves when handling it or the gels, buffers and chambers it has been in contact with, it's best practice to have a dedicated work area for ethidium bromide. Dispose of hazardous chemicals according to your institution's chemical waste guidelines.

Before start

Have the RNA sample ready on ice or in a benchtop cooler.

Gel preparation (for 100 mL of a 1% agarose TAE+Bleach gel)

1h 16m

1 **For 100 mL of a 1% agarose gel, add 1 g agarose to a glass flask with 300-500 mL capacity.** 3m

2 **Add 99 mL of 1× TAE buffer to the flask and add a stirring bar.** 2m

3 **Add 1 mL of ChloroxPro Germicidal Bleach. Mix on a stir plate for 5 min at a low speed.** 5m
This mixing is only for the bleach as the agarose will only dissolve under heat.

4 **Take a wettened, crumbled paper towel and use it to loosely plug the flask.** 1m
This just helps with overboiling and evaporation in the next step.

5 **Microwave at a medium setting for 5 min.** 6m
This helps prevent the liquid from boiling over. Higher power might dissolve the agarose quicker but requires constant attention and intervention.

6 **Carefully take out the hot flask and check that the agarose has dissolved completely.** 1m
For higher agarose percentages, additional 1 or 2 min might be required to fully dissolve the agarose.

7 **Add a stirring bar and place on a stirring plate to mix and let cool down to ~60°C for ~20 min.** 20m
The stirring speed should be high enough to fully mix the solution but not high enough to introduce air bubbles.

8 **OPTIONAL: Add dye for RNA detection (e.g., ethidium bromide) to the desired concentration (*e.g.*, add 50-100 µL from a 10 mg/mL ethidium bromide solution for 100 mL gel).** 1m

9 **Pour the gel into an appropriate gel tray with a fitting comb and let it solidify for 30 min.** 35m
This can be accelerated if the gel tray is placed at 4°C.

10 **Use gel immediately or store moisture-sealed at 4°C for a few days.** 2m

Sample preparation

20m

11 **For a 1-µL RNA sample in a PCR tube, add 4 µL nuclease-free water and 1 µL 6× DNA loading dye.** 1m

1 µL of RNA is plenty for highly concentrated in vitro *transcribed RNA, for lower concentration, increase the volume and decrease the water volume.*

12 **Transfer 6 µL of an appropriate DNA ladder (ideally in the same DNA loading dye) into another PCR tube.** 1m

13 **Denature sample and ladder at 70°C for 10 min in a PCR cycler.** 12m
This denaturing step helps with resolving at least some secondary structures, so in the best case, the RNA runs in a single band, but multiple bands are still a possibility.

14 **Immediately cool down to 4°C in the cycler or on ice for 5 min and keep at that temperature until loading the gel.** 6m

Sample loading and running of the gel 1h 9m

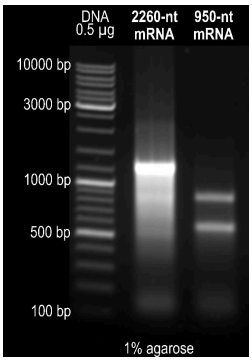
15 **For cleanest results fill the electrophoresis tank with fresh 1×TAE.** 2m
The bleach in the gel prevents RNA degradation so no special precautions need to be taken and the gel can be run in the same tank as DNA.
If using ethidium bromide for RNA detection without post-run staining, add 50 µL per liter buffer at the anode of the tank.

16 **Load all of the denatured ladder and sample into the wells.** 2m

17 **Run the gel at a constant 3-5 V/cm for 20-30 min.** 30m
Higher percentage gels need longer for a good separation.

18 **OPTIONAL: For highest quality images or if no ethidium bromide (or alternative dyes) were added to the gel and buffer, do your routine gel staining/destaining procedure (depending on the dye used, same as for DNA gels).** 30m

19 **Image gel and check for signs of RNA degradation (marked smears below the bands or blobs on the bottom of the gel, see example below).** 5m



Example of two (partially) degraded RNA samples on a 1% agarose, TAE+bleach gel.

Note

The DNA ladder gives only a rough estimate of the actual size, the bands of the RNA sample might be lower or higher than the corresponding ladder band and there might be multiple stacked bands if the RNA has strong secondary structures which were not resolved during the denaturation. If a more precise size estimation is needed, use the RNA electrophoresis protocol with RNA-denaturing loading dye or switch to fully denaturing protocols involving formaldehyde, formamide or glyoxal/DMSO.

Protocol references

This protocol is based on the following one and some more technical details and precautions:
Aranda PS, LaJoie DM, Jorcyk CL. 2012. Bleach gel: a simple agarose gel for analyzing RNA quality. ELECTROPHORESIS. 33(2):366–369. doi:10.1002/elps.201100335. https://doi.org/10.1002/elps.201100335

The sodium borate electrophoresis buffer is based on:
Brody JR, Kern SE. 2004. Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. BioTechniques. 36(2):214–216. doi:10.2144/04362BM02. https://doi.org/10.2144/04362BM02