Making and running agarose gels

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Agarose gel electrophoresis is a simple, versatile technique for resolving DNA (*main protocol*), RNA (*Alternative A*), and large protein complexes (*Alternative B*) for analytical or preparative purposes. Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE) buffers provide reliable separation of DNA fragments, with TBE favoring small fragments and TAE better suited for larger fragments and downstream enzymatic recovery. However, Tris-based buffers gradually lose buffering capacity during electrophoresis, causing heating and potential gel distortion at high voltages. Alternative electrolytes, such as sodium borate (borax) or lithium acetate, allow faster electrophoresis at higher voltages with lower heat buildup.

This is a bench card. Full protocol available online.

Procedures

›› *Choosing an electrophoresis buffer for DNA separation*

(1.) Select running buffer, agarose concentration, and voltage based on the DNA fragment size and goals:

Purpose	DNA size range	Agarose content	Running buffer	Voltage	Staining
Analytical separation	<1 kbp	1.0–1.8%	0.5 × TBE	10–15 V/cm	Post-run staining
	1–12 kbp	0.7–1.0%	0.5 × TBE	5–10 V/cm	Post-run staining
	>12 kbp	0.6–0.8%	1 × TAE	5 V/cm	Post-run staining
Preparative recovery	<1 kbp	1.2–2.0%	1 × TAE	10–15 V/cm	In-gel
	1–12 kbp	0.8–1.2%	1 × TAE	5–10 V/cm	In-gel
	>12 kbp	0.6%	1 × TAE	5 V/cm	In-gel
Speed/Throughput	<1 kbp	1.0%	0.5 × Tris-borate	20–25 V/cm	In-gel
		1.0%	1 × Borax	30 V/cm	In-gel
	1–12 kbp	1.0%	1 × Lithium-acetate	30 V/cm	In-gel

(2.) Choose a DNA stain according to sensitivity needs:

Stain	Detection limit	Wavelength	Working concentration
Ethidium bromide	1–5 ng per band	UV light	0.2–0.5 mg/L
SYBR® Safe	100 pg per band	UV *or* Blue light	1 : 10,000
Thiazole Orange (TO)	50–100 pg per band	Blue light	1 : 10,000
GelRed®	50 pg per band	UV *or* Blue light	1 : 10,000
SYBR® Gold	10–25 pg per band	Blue light	1 : 10,000

›› *Casting an agarose gel*

- ☐ Erlenmeyer flask *or* Screw-cap bottle
- ☐ Stir bar and plate
- ☐ Microwave oven
- ☐ Agarose, low EEO (LE)
- ☐ Running buffer

(1.) Choose a heat-resistant container two- to four-times the intended gel volume. Add the electrophoresis buffer and a magnetic stir bar.

(2.) Slowly sprinkle the agarose powder into the stirred buffer to prevent clumping.

> ***Critical:*** Use low electroendosmosis (EEO) agarose for better mobility, sharper separation, and minimized electro-osmotic backflow during electrophoresis.

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(3.) *Optional:* Soak the agarose for 15 min in running buffer to reduce foaming during heating, especially for concentrations above 3%. 15 min

(4.) Weigh the container and record the weight to monitor evaporation losses after heating.

(5.) Cover the container loosely: seal with pierced plastic wrap or leave the screw cap loosened by one turn.

> ***Safety:*** Allowing some ventilation prevents pressure buildup while minimizing excessive evaporation during microwaving.

(6.) Heat the agarose suspension in the microwave at Medium power for 1–2 min. Remove, swirl gently, and repeat as needed until almost dissolved.

> ***Safety:*** Agarose solutions can become superheated. Swirl gently to prevent sudden boiling over. Point the opening of the flask away from you and others while swirling! If in doubt, let the solution rest for a couple of minutes.

(7.) Heat briefly at High power until the solution comes to a rolling boil. Hold at boiling for 30–60 s to fully dissolve any remaining particles. Swirl gently after heating.

(8.) Re-weigh the container. If weight loss exceeds 5%, add hot distilled water to compensate for evaporation. Mix thoroughly.

(9.) Cool the molten agarose to 45–60 °C before casting.

(10.) *Optional:* Add DNA staining dye to the gel as appropriate.

> ***Critical:*** Do not include SYBR® Green Stains in the agarose solution: the bands will start to smear (overloading) and alter the electrophoretic separation.

(11.) Prepare the casting tray. Position the comb so that the teeth are suspended 1–2 mm above the tray base.

(12.) Pour the molten agarose smoothly into the tray in one continuous motion to a thickness of 3–4 mm.

> ***Quality assurance:*** Avoid overfilling beyond the comb teeth. Thin gels improve resolution, reduce heating artifacts, and allow faster post-staining penetration.

(13.) Allow the gel to solidify undisturbed for 15–20 min at room temperature. 15–20 min

(14.) Once solidified, place the gel with the tray into the gel tank. Cover 1–2 mm deep in running buffer. Carefully remove the comb by pulling straight upward without tilting.

›› *Loading samples*

- ☐ R0177 6 × Gel loading buffer, 40 µL
- ☐ R0182 25 mg/L DNA ladder, 4–8 µL
- ☐ 20 × Gel loading dye, 12 µL

(1.) *Optional:* To supplement the 6 × loading buffer with a loading dye, add 0.3 vol 20 × loading dye for a 5 × loading buffer with dye. The dye front will migrate at:

	Agarose content				
DNA dye	**0.7%**	**1.0%**	**1.5%**	**2.0%**	**3.0%**
Xylene cyanol	8 000 bp	4 000 bp	2 000 bp	900 bp	400 bp
Cresol red	3 000 bp	1 500 bp	900 bp	300 bp	100 bp
Bromophenol blue	600 bp	400 bp	240 bp	120 bp	25 bp
Orange G	100 bp	50 bp	20 bp	10 bp	5 bp

(2.) Choose the appropriate molecular weight marker.

(3.) Mix each DNA sample with loading buffer:

- Add 2.0 µL of 6 × loading buffer per 10 µL sample, or
- Add 2.5 µL of 5 × loading buffer (pre-mixed with tracking dye) per 10 µL sample.

> ***Quality assurance:*** Aim to load between 10–100 ng DNA per band for ethidium bromide staining. For more sensitive dyes like SYBR® Gold, adjust accordingly. If you are unsure about how much DNA is in your sample, load varying amounts per lane.

(4.) Load the gel by gently lowering the pipette tip just into the well opening. Dispense the sample slowly without puncturing the gel bottom. Steady the pipette with one finger of your non-pipetting hand if needed. Change pipette tips between different samples.

›› *Running the gel*

(1.) Connect the electrophoresis unit: the negative electrode (cathode, black) must be closest to the wells; the positive electrode (anode, red) opposite the wells. Remember: “Run towards red!”

(2.) Apply voltage according to the task:

- For analytical gels: 5–10 V/cm between electrodes.
- For preparative gels: 5 V/cm for better resolution and minimal heating.

(3.) Stop the run when the band of interest has migrated 40–60% of the gel length or when desired separation is achieved.

⊞ Optional: *Post-run staining of DNA*

- ☐ R0018 10 g/L Ethidium bromide
- ☐ R0183 10 000 × Thiazole orange, 2 µL

(1.) Immerse the gel for 15–20 min in 1 × staining solution with gentle agitation if available. 15 min

> ***Safety:*** Always handle ethidium bromide and DMSO-based dyes with gloved hands in a designated area. Collect staining waste separately for proper disposal.

(2.) *Optional:* Rinse the gel three times with deionized water to reduce background fluorescence.

A › *Separation of RNA in agarose gels*

- ☐ R0172 Water, 100 mL
- ☐ 1.3 × RNA sample buffer
- ☐ R0184 5 × MOPS-acetate running buffer, 0.5 L

(1.) For RNA separation, use a 1–1.5% agarose gel in 1 × MOPS-acetate buffer containing 2.2 M (6.5%, w/v) formaldehyde.

> ***Critical:*** RNA is highly sensitive to degradation. Use RNase-free reagents and equipment, including DEPC-treated water. Wear gloves at all times.

> ***Safety:*** Formaldehyde is a known carcinogen. Cast the gel in a chemical fume hood, and allow to set for at least 30 min at room temperature. Melt agarose in DEPC-treated water, before combining with 5 × MOPS-acetate buffer and formaldehyde.

(2.) Combine 1.0 vol of RNA sample containing up to 30 µg per lane with 3 vol 1.3 × RNA sample buffer. Incubat for 15 min at 65 °C and chill on ice.

(3.) Mix with 0.1 vol RNA loading dye.

(4.) Pre-run the gel for 5 min at 5 V/cm. Immediately load the sample.

(5.) Run at 3–4 V/cm in 1 × MOPS-acetate running buffer until appropriate separation is achieved.

> ***Quality assurance:*** Collect the running buffer every 1–2 h from the reservoir, mix, and return to the gel apparatus.

(6.) Stain RNA with ethidium bromide or SYBR® Green II.

(7.) *Optional:* Proceed with Northern blotting.

B › *Separation of proteins in agarose gels*

(1.) Prepare a low electroendosmosis (EEO) agarose gel. Adjust concentration depending on target size:

Protein size range	Agarose content	Agarose type
20–200 kDa	5%	MetaPhor®, NuSieve®
150–300 kDa	3%	MetaPhor®, NuSieve®
300–600 kDa	2%	MetaPhor®, NuSieve®
>600 kDa	1–1.5%	SeaKem®, SeaPlaque®

(2.) Use 1 × Tris-borate (or Tris-Gly running buffer SOP 0007 to cast a horizontal agarose gel.

> ***Critical:*** Do not add SDS to the gel buffer to avoid excess foaming during agarose dissolution. Only add SDS to the running and sample buffer for denaturing electrophoresis.

(3.) Load proteins with loading buffer containing bromophenol blue or xylene cyanol FF as tracking dye.

(4.) Run at 10–20 V/cm for 3–4 h until the tracking dye travels to the bottom of the gel. 3–4 h

(5.) Stain proteins with Coomassie Brilliant Blue SOP 0010, Silver stain SOP 0011, Amido black, Violet 17, or commercial protein stains such as SERVA Blue after electrophoresis.

(6.) *Optional:* To recover proteins, use low-melting agarose and extract bands by freeze-thaw or gentle buffer diffusion.

List of references

Recipe (available online) Troubleshooting (available online) Notes (available online)