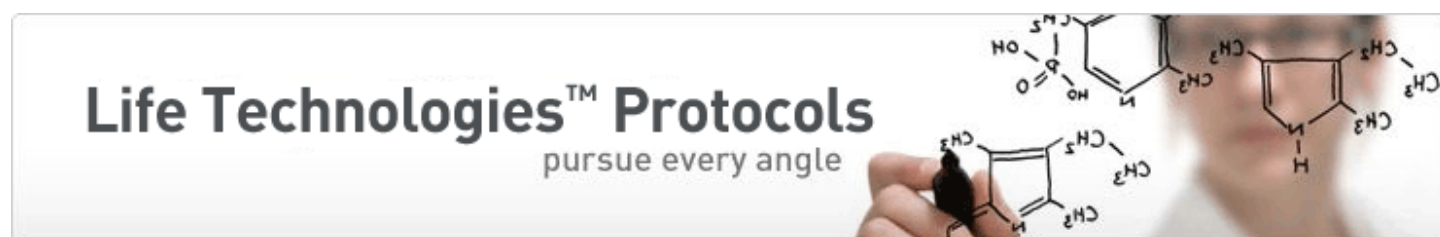


DNA Analysis Protocols

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- [CloneChecker™ System](#)
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UltraPure™ Agarose



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Introduction

UltraPure™ Agarose is a standard melting temperature, multi-purpose agarose that is ideal for routine separation analysis. UltraPure™ Agarose resolves DNA and RNA fragments from 500–23,000 bp, and has no detectable DNase or RNase activity. It can also be used for:

- Analytical separation of DNA, RNA, and PCR fragments
- Recovery of DNA, RNA, and PCR fragments
- Southern and northern blotting of fragments
- Ouchterlony and radial immunodiffusion (RID)

Specifications

1.5% Conc.

Gel Strength*	≥ 1,200 g/cm ²
Gel Point	≤ 36°C
Melting Point	≥ 90°C

* Gel strength is calculated at 1% concentration.

Usage

Refer to the table below for the recommended concentration of UltraPure™ Agarose needed to resolve DNA fragments of the approximate listed range:

Fragment Size	% Agarose (in 1X TAE)	% Agarose (in 1X TBE)
1,000–20,000	0.60	0.50
800–10,000	0.80	0.70
400–8,000	1.00	0.85
300–7,000	1.20	1.00
200–4,000	1.50	1.25
100–3,000	2.00	1.75

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Dissolving UltraPure™ Agarose

Method 1: Microwave

1. Determine the amount of agarose solution needed to cast your gel.

Note: Remember to take the thickness of the gel into account, as it affects both well volume and power requirements.
2. Add room temperature buffer (TAE or TBE) into a flask that can hold 2–4 times the volume of your agarose solution. Place a magnetic stir bar into the flask.
3. Put the flask on a magnetic stirrer and slowly sprinkle the required amount of agarose powder into the flask as the solution mixes, to prevent the formation of agarose clumps.
4. Remove the stir bar.
5. Weigh the flask and solution before heating.
6. Cover the mouth of the flask with plastic wrap, and pierce the wrap with a small hole for ventilation.
7. Place the flask in the microwave oven and heat the solution until bubbles appear.
8. Remove the flask carefully, and swirl gently to resuspend any agarose particles. Exercise caution – microwaved solution may become superheated and foam over when agitated.
9. Reheat the solution until the solution comes to a boil, and all the agarose particles are dissolved.
10. Remove the flask carefully and swirl gently to mix the solution.
11. Place the flask on a scale, and bring it back to its initial weight (from Step 5) with warm distilled water.
12. Mix gently and cool to 50–60°C (at room temperature for at least 20 minutes) before pouring the solution into the

casting tray.

Method 2: Boiling water bath

1. Determine the amount of agarose solution needed to cast your gel. **Note:** Remember to take the thickness of the gel into account, as it affects both well volume and power requirements.
2. Add room temperature buffer (TAE or TBE) into a flask that can hold 2–4 times the volume of your agarose solution. Place a magnetic stir bar into the flask.
3. Put the flask on a magnetic stirrer and slowly sprinkle the required amount of agarose powder into the flask as the solution mixes, to prevent the formation of agarose clumps.
4. Weigh the flask and solution before heating.
5. Cover the mouth of the flask with plastic wrap, and pierce the wrap with a small hole for ventilation.
6. Bring the solution to a boil while stirring, and allow it to boil gently for approximately 10 minutes or until the agarose is completely dissolved.
7. Place the flask on a scale, and bring it back to its initial weight (from Step 4) with warm distilled water.
8. Mix gently and cool to 50–60°C (at room temperature for at least 20 minutes) before pouring the solution into the casting tray.

Visualization of DNA

For visualization of DNA in the gel, a fluorescent dye can be added to the agarose solution just prior to pouring, or the gel can be stained after electrophoresis. For the intercalating dye ethidium bromide, use a final concentration of 0.5 µg/ml. If more sensitive detection is required, use SYBR® Green I nucleic acid gel stain (Invitrogen Cat. no. S-7563), or SYBR® Safe DNA gel stain (Invitrogen Cat. no. S33102). Use of SYBR® Safe DNA gel stain in conjunction with blue light transillumination is recommended for gel extraction procedures for cloning purposes. Refer to the appropriate instructions for these products for in-gel staining, or post-staining protocols.

Dye Mobility

Refer to the following table for the migration of Bromophenol Blue and Xylene Cyanol tracking dyes in relation to DNA:

% Agarose	Bromophenol Blue	Xylene Cyanol		
	TAE	TBE	TAE	TBE
0.3	2,900	2,850	24,800	19,400
0.5	1,650	1,350	11,000	12,000
0.75	1,000	720	10,200	9,200
1	500	400	6,100	4,100
1.25	370	260	3,560	2,500
1.5	300	200	2,800	1,800
1.75	200	110	1,800	1,100
2	150	70	1,300	850

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