Agaroses

Code	Description	Size
0710-25G	**Agarose I™**	25 g
0710-100G	**Agarose I™**	100 g
0710-250G	**Agarose I™**	250 g
0710-500G	**Agarose I™**	500 g
K857-100TABS	**Agarose I™ Tablets**	100 tablets
K857-1000TABS	**Agarose I™ Tablets**	1000 tablets
0815-25G	**Agarose II™**	25 g
0815-100G	**Agarose II™**	100 g
0815-250G	**Agarose II™**	250 g
X174-25G	**Agarose LF™**	25 g
X174-100G	**Agarose LF™**	100 g
X174-250G	**Agarose LF™**	250 g
E776-25G	**Agarose 3:I HRB™**	25 g
E776-100G	**Agarose 3:I HRB™**	100 g
E776-250G	**Agarose 3:I HRB™**	250 g
J234-3G	**Agarose SFR™**	3 g
J234-25G	**Agarose SFR™**	25 g
J234-100G	**Agarose SFR™**	100 g
J234-250G	**Agarose SFR™**	250 g
N605-25G	**Agarose RA™**	25 g
N605-100G	**Agarose RA™**	100 g
N605-250G	**Agarose RA™**	250 g
N605-500G	**Agarose RA™**	500 g
N605-1KG	**Agarose RA™**	1 kg
N605-Q-3G	**Agarose RA™**	3 g

Directions for Use

General Information

VWR Life Science AMRESCO offers a range of agaroses formulated to meet rigorous criteria for the separation of biological molecules by gel electrophoresis. The exceptional purity and uniform particle size of VWR Life Science AMRESCO agaroses provide superior clarity, maximal resolution and reproducible performance with each run. All agarose products are guaranteed to be free of DNase, RNase and protease.

The appropriate agarose for a given application is dependent on a variety of factors, including the type of nucleic acid, whether it is single stranded (ss) or double stranded (ds), its size and use in downstream applications. See Table 1 for a detailed application guide to VWR Life Science AMRESCO agaroses.

- Agarose RA™ - Routine Analysis
- Agarose I™ - General Purpose
- Agarose II™ - Low Melt
- Agarose SFR™ - Super Fine Resolution, Low Melt
- Agarose 3:3 HRB™ - High Resolution Blend
- Agarose LF™ - Low EEO/High Gel Strength for Pulse Field

Storage/Stability

Store at room temperature (18 to 26°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Table 1

Description	Code	Optimal Seperation Range (bp)	Specifications (1.5% gel)					Recommended Applications								
			Gelling Range (°C)	Melting Range (°C)	Gel Strength (g/cm²)	EEO (-Mr)	Sulfate (%)	All Purpose	PCR & Cloning	High Resolution	Low Melt	Preparative Gels	Northern Blotting	Southern Blotting	Pulsed-Field Gel Electrophoresis	In-gel PCR / In-gel Digestion
Agarose I™	0710	250 - 22,000	36 - 39	87 - 89	≥ 1,200	< 0.13	≤ 0.15	√	√			√	√	√		
Agarose II™	0815	100 - 20,000	24 - 29	62- 68	≥ 250 *1% gel*	≤ 0.12	≤ 0.10		√		√					√
Agarose RA™	N605	250 - 22,000	34 - 38	87 - 89	≥ 1,200	< 0.13	≤ 0.15	√								
Agarose SFR™	J234	20 - 2,000	≤ 30	≤ 70	≥ 500	≤ 0.12	≤ 0.11		√	√	√	√				√
Agarose 3:1 HRB™	E776	50 - 2,000	34 - 38	85 - 89	≥ 2,000	≤ 0.12	≤ 0.10			√			√	√		
Agarose LF™	X174	1,000 - 40,000	37 - 41	93 - 96	≥ 2,000	≤ 0.06	< 0.06						√	√	√	

Additional Required Materials Not Supplied

- TAE or TBE buffer
- Deionized, distilled water
- Sample loading buffer
- Visualization dye
- Gel documentation system
- Microwave or heating/magnetic stirrer plate and stir bar, or autoclave
- Gel electrophoresis unit, casting stand, power supply

Protocol/Procedure:

Microwave instructions

Recommended for agarose concentrations ≤ 3%.

1. Determine gel volume and agarose concentration. Volume = surface area of the casting chamber X gel depth. Optimal resolution is usually obtained on gels 3-4 mm thick.

2. In a microwavable container 2 – 4 times the desired gel volume, slowly add agarose powder to 1X buffer solution (TAE or TBE) while gently swirling the container. If using Agarose I™ Tablets, crush the tablet into a powder with a clean rod or spatula and mix with the buffer.
3. Weigh the flask containing the buffer and agarose.
4. Heat the buffer with uniformly dispersed agarose in a microwave on high power for 30 seconds, or until bubbles appear. (*Heating times may vary depending on microwave wattage, gel volume and agarose concentration*.)
    - **Caution:** Handle the hot flask very carefully. Microwaved solutions may become superheated and boil over when moved or touched.
5. Remove the container from the microwave and gently swirl the agarose solution to resuspend any particles.
6. Heat the solution at high power another 30 seconds, or until the solution comes to a boil.
7. Repeat the gentle swirling of the agarose solution.
8. Heat the solution on high power until all particles are dissolved (approximately 10-35 seconds).
9. Remove the boiling solution from the microwave oven and allow to cool briefly (1-2 minutes) at room temperature. Gently swirl the solution to release entrapped air bubbles. If desired, a visualization dye, such as EZ-Vision® In-Gel Solution, 10,000X or ethidium bromide, may mixed into the agarose solution.
10. Reweigh the container to determine the loss of water by evaporation. Add sufficient hot distilled water to obtain the original weight. Gently swirl the solution.
11. Allow the agarose solution to cool at room temperature to approximately 50-60°C before casting the gel.

Hot Plate / Magnetic Stirrer Instructions

Recommended for all agarose concentrations, especially high concentrations between 4-5%.

1. Determine gel volume and agarose concentration. Volume = surface area of the casting chamber X gel depth. Optimal resolution is usually obtained on gels 3-4 mm thick.
2. To a heat-resistant container that is 2-4 times the desired gel volume, , slowly add agarose powder to 1X buffer solution (TAE or TBE) while rapidly stirring with a Teflon coated stir bar. If using Agarose I™ Tablets, crush the tablet into a powder with a clean rod or spatula and mix with the buffer.
3. Weight the flask containing the buffer and agarose.
4. Heat the solution with rapid stirring until it boils.
5. Maintain a gentle boil until the agarose is completely dissolved, about 3-10 minutes. Inspect the solution for agarose crystals and continue boiling if necessary.
6. Remove the solution from the hot plate and allow to cool briefly (1-2 minutes) at room temperature. Gently swirl the solution to release entrapped air bubbles. If desired, a

visualization dye, such as EZ-Vision® In-Gel Solution, 10,000X or ethidium bromide, may mixed into the agarose solution.

7. Reweigh the container to determine the loss of water by evaporation. Add sufficient hot distilled water to obtain the original weight. Gently swirl the solution.
8. Allow the agarose solution to cool at room temperature to approximately 50-60°C before casting the gel.

Autoclave Instructions

Highly recommended for agarose concentrations > 5%, but may be used for lower concentrations as well. In order to avoid contamination of the autoclave, do not autoclave agarose solutions containing ethidium bromide or other mutagenic intercalating stains.

1. Determine gel volume and agarose concentration. Volume = surface area of the casting chamber X gel depth. Optimal resolution is usually obtained on gels 3-4 mm thick.
2. In an autoclavable container 2-4 times the desired gel volume, slowly add agarose powder to 1X buffer solution (TAE or TBE) while gently swirling the container. If using Agarose I™ Tablets, crush the tablet into a powder with a clean rod or spatula and mix with the buffer.
3. Weigh the flask containing the buffer and agarose.
4. Cover the flask with aluminum foil and autoclave at 121°C for 15 minutes.
5. Carefully remove the solution from the autoclave and allow to cool briefly. If desired, a visualization dye, such as EZ-Vision® In-Gel Solution, 10,000X or ethidium bromide, may mixed into the agarose solution.
6. Reweigh the container to determine the loss of water by evaporation. Add sufficient warm distilled water to obtain the original weight. Gently swirl the solution.
7. Allow the agarose solution to cool at room temperature to approximately 50-60°C before casting the gel.

Gel Casting

1. Assemble the gel casting stand according to the manufacturer's instructions.
    - Ensure casting stand is level.
    - Ensure comb teeth are free of dried agarose.
2. When agarose solution cools to ~55°C, gently swirl to help dissipate most of the remaining air bubbles.
3. Pour the gel into the prepared casting unit to a depth of 3-4 mm. Immediately after pouring, insert comb and remove any air bubbles that may be trapped under or between the teeth of the gel comb.
4. Allow the gel to completely solidify at room temperature, approximately 30-45 minutes. If using Agarose SFR™, optimal resolution will be achieved if the gel is subsequently placed at 4°C for 30 minutes prior to electrophoresis.

Electrophoresis

1. Once the agarose gel is solidified, flood it with running buffer and carefully remove the comb and casting dams.
2. Place the gel in the electrophoresis chamber and add running buffer (TAE or TBE) until the gel is submerged under 3-5 mm of buffer. Avoid adding excessive buffer, as larger volumes will retard migration of samples and generate band distortion through reduction of the voltage gradient.
3. Carefully flush the sample wells with buffer to remove loose gel fragments.
4. Prepare samples by mixing with sample loading buffer to a final concentration of 1X.
5. Load samples by pipette, taking care to not puncture the sample well.
6. Attach the gel box cover and power leads and apply current to the unit. Bubbles will appear around the electrodes when current is flowing. The amount of voltage to apply is determined by the distance between the electrodes.
    - **Note:** To obtain optimal resolution with Agarose SFR™, apply 3-4 V/cm. If higher voltage is desired, run gel at 4°C.

Optimal Voltages for Agarose Gel Electrophoresis			
DNA Fragment Size (bp)	**Voltage (V/cm)**	**Buffer for Preparative Gel**	**Buffer for Analytical Gel**
≤ 1 kb	5	TAE	TBE
1 kb – 12 kb	4 – 10	TAE	TAE/TBE
> 12 kb	1 – 2	TAE	TAE

7. Turn off power supply when samples have resolved sufficiently. Sample migration may be monitored by progression of tracking dye(s) included in the sample loading buffer. Optimal resolution and band sharpness are obtained within the upper 60% of the gel.
8. Visualize bands using an appropriate staining technique, such as EZ-Vision® In-Gel Solution or ethidium bromide post-staining. Note that post-staining is unnecessary if nucleic acid visualization dye was added directly to the gel or included in the sample loading buffer.

Frequently Asked Questions

Problem	Cause	Solution
Why are there clumps in the gel?	Insufficient dispersion of agarose	Increase agitation while slowly adding agarose to the buffer at room temperature.
		Increase dispersion time by keeping the solution at room temperature for 1-5 minutes before heating.
		Keep agarose dispersed during initial heating in the microwave. After 30 seconds of microwave heating, remove flask and swirl to resuspend crystals. Repeat this step before bringing the solution to a boil.
Why are there bubbles in the gel?	Air bubbles trapped in molten agarose	After the boiled agarose solution cools to 50-55°C, gently swirl the solution before pouring into the gel casting stand.
Why does the gel overheat or melt during electrophoresis?	Excessive buffer depth	Do not exceed a buffer overlay of 3-5 mm.
	Buffer depletion	TBE has greater buffering capacity at the initial neutral pH, since the pK_a of borate is closer to the initial pH than that of acetate.

		Mini-electrophoresis chambers experience buffer depletion within 10-13 Watt hours.
		Standard electrophoresis chambers (1.5-2 liter capacity) experience buffer depletion in 40-50 Watt hours. Consult the chamber manufacturer for specific instructions.
		If the run is performed for extended periods in TAE, it may be necessary to recirculate the buffer to prevent development of a pH gradient. Monitor the pH in anode and cathode chambers during electrophoresis to ensure that depletion is not occurring.
Why are the bands faint or invisible?	Insufficient sample loaded	Increase sample amount loaded. For DNA, sharp bands are obtained by loading no more than 50-100 ng per DNA band. For RNA, load a maximum of 30 µg total RNA per lane.
	Degraded sample	Use nuclease-free reagents during sample preparation.
	Samples migrated off the gel	Increase the gel concentration.

		Reduce electrophoresis time.
		Lower the voltage.
		Closely monitor tracking dyes included in the sample loading buffer.
Why are the bands smearing, smiling or distorted?	Sample loading volume is too large	Reduce the sample volume.
	Voltage too high	Voltage should not exceed 20 V/cm and temperature should remain < 30°C.
	Overloaded DNA	50-100 ng/band is generally the maximum amount that can generate sharp bands.
	Excessive buffer depth Buffer depletion Degraded sample	See above
	Excess salt in sample	Remove excess salt by ethanol precipitation.
	Protein contamination	Remove protein contamination by phenol extraction.
	Very large DNA fragments	Run gel at low field strength (1-2 V/cm)
		Use Agarose LF™ with TAE Buffer.
	Low molecular weight band diffusion	Increase the gel concentration.

		Use TBE buffer for analytical applications.
		Run gel at 4-10 V/cm.
		Switch to Agarose 3:1 HRB™ or Agarose SFR™.
	Sample creeps up side of wells prior to applying current	Use a loading buffer containing Ficoll® as a density agent instead of glycerol.
	Uneven gel pores	Allow agarose solution to cool at room temperature to ~50-55°C before pouring to obtain a more uniform pore size.

For Technical Support

Toll Free: 1-800-610-2789 (USA & Canada)
Fax: (440) 349-0235
Email: techinquiry@amresco-inc.com

AMRESCO, LLC
A VWR Company

Corporate Headquarters
28600 Fountain Parkway
Solon, Ohio USA 44139-4300

Tel: 440/349-1199
Fax: 440/349-1182
www.amresco-inc.com

Agaroses

ZY0582
Rev. 2 11/2015