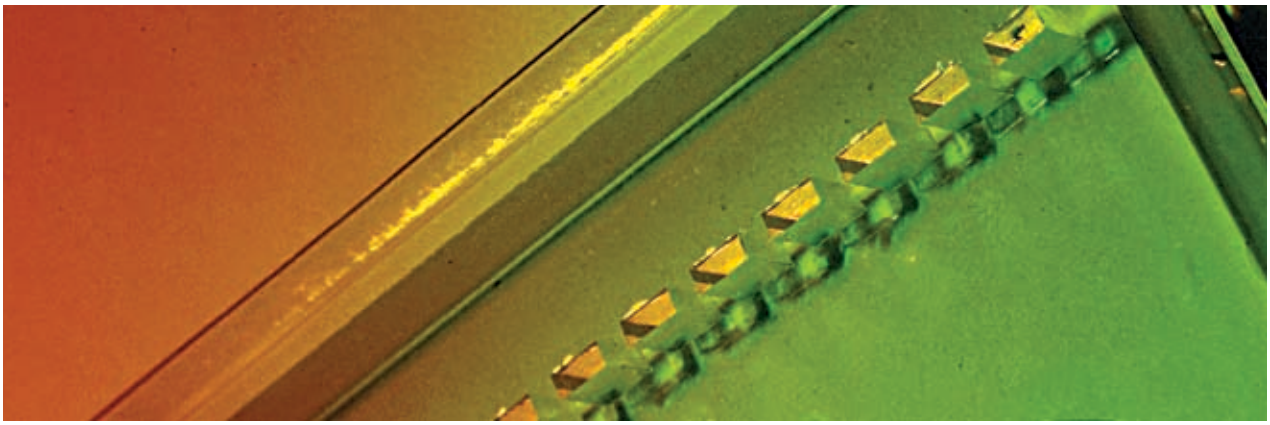
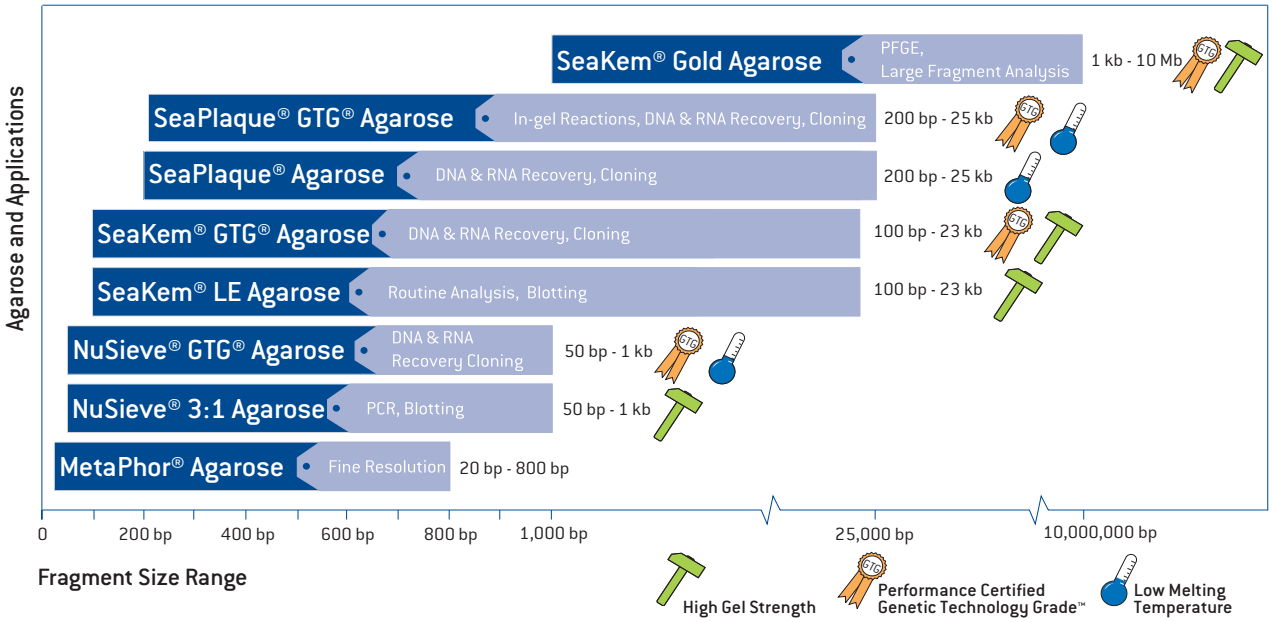


In This Section

Agarose Selection Guide

Selecting the best agarose for your application can minimize opportunity for error, optimize results, and even reduce cost. Lonza offers a wide range of agarose types that are specifically engineered to optimize results by fragment size, sample type and application. The selection tools below will get you started. The following pages will guide you to the right concentration, buffer and marker to use for best performance in your experiment.

Choose the Agarose that is Right for You



Agarose and Compatible Techniques

Recovery Method	SeaKem® LE	SeaKem® GTG®	SeaPlaque®	SeaPlaque® GTG®	NuSieve® 3:1	NuSieve® GTG®	MetaPhor®	SeaKem® Gold	SeaPrep®	InCert®	I.D. NA®
In-Gel Reactions				■		■					
ß-Agarase			■	■		■					
Phenol/Chloroform			■	■		■					
Recovery Columns	■	■	■	■	■	■	■	■			■
Electroelution	■	■	■	■	■	■	■	■			■
Freeze/Squeeze	■	■	■	■	■	■	■	■			■
Blotting											
Southern <1 kb					■						
Southern >1 kb	■										
Northern <1 kb					■						
Northern >1 kb	■										
Specialty Applications											
Viral Plaque Assays			■	■							
Preparation of Megabase Samples										■	
PFGE								■		■	
Cell Culture			■	■			■				
Encapsulation & Embedding of Cells									■		
DNA Identity Testing											■
Comet Assays	■	■									

Our agarose is GUARANTEED DNase/RNase Free

Introduction

Nucleic acid applications take advantage of the specific properties of different agaroses and derivatized agaroses. The appropriate choice of agarose depends on the size of the DNA to be analyzed and any subsequent manipulations required. Gelling/melting temperatures, electroendosmosis and gel strength are all important factors in choosing the right agarose for your application. Refer to page 79 for analytical specifications of Lonza agarose.

Genetic Technology Grade™ (GTG®) Agaroses

Our Genetic Technology Grade™ (GTG®) Agarose Products are specially prepared and certified for demanding molecular biology applications for nucleic acids, including PCR amplified products. Lonza's GTG® Agarose quality tests go beyond standard assays such as, DNase and RNase testing, to include enzymatic performance measurements. Our additional testing provides a more realistic index of overall product quality and reliability. You no longer need to screen agarose lots to find those that yield biologically active DNA.

- **The following agaroses are GTG® Certified:**
  - SeaKem® GTG® Agarose
  - SeaPlaque® GTG® Agarose (low melting temperature agarose)
  - NuSieve® GTG® Agarose (low melting temperature agarose)
  - SeaKem® Gold Agarose
- **Lonza performs the following tests on GTG® Certified Agaroses:**
  - DNA binding
  - DNase and RNase activity
  - DNA resolution
  - Gel background-gel exhibits low background fluorescence after ethidium bromide staining
  - In-gel cloning (low melt agarose)
  - In-gel restriction digestion (low melt agarose)
  - Restriction-ligation assay (SeaKem® GTG®)

Molecular Biology Grade Agaroses

Molecular biology grade agaroses are suitable for general analytical separation of DNA.

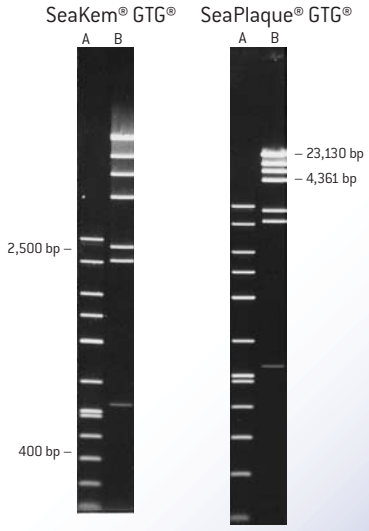
- **The following agaroses are considered molecular biology grade agaroses:**
  - MetaPhor® Agarose
  - SeaKem® LE Agarose
  - NuSieve® 3:1 Agarose
  - SeaPlaque® Agarose (low melting temperature agarose)
- **Lonza screens our molecular biology grade agaroses for the following parameters:**
  - DNA binding
  - DNase and RNase activity
  - Gel background staining

FDA Listing

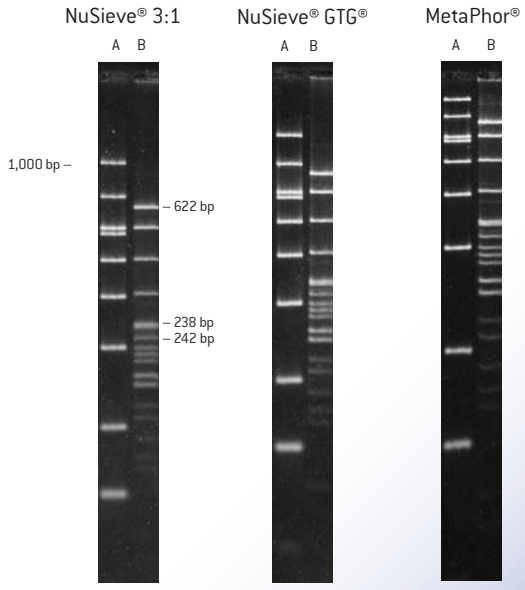
Lonza agarose types are listed as Class 1 Medical Devices under registration number 1219614.

DNA resolution examples

The photographs below show the different resolution properties of Lonza agaroses.



Separation of DNA markers in 1% SeaKem® GTG® and SeaPlaque® GTG® Agarose gels in 1X TBE Buffer (Prepared from AccuGENE® 10X TBE Buffer).
Lane A: Lonza's 50 - 2,500 bp DNA Marker (~0.25 ng/band)
Lane B: New England BioLabs *Hind* III digest of lambda DNA (0.125 mg/lane) 20 cm long gels were run at 6 V/cm for ~ 2.5 hrs. Gels were post stained using Lonza's 1X GelStar® Nucleic Acid Gel Stain for 30 minutes. No destain.



Resolution Performance of Lonza agaroses for DNA <1 kb
Separation of DNA markers in 3% NuSieve® 3:1, NuSieve® GTG® and MetaPhor® Agarose gels in 1X TBE (Prepared from AccuGENE® 10X TBE Buffer).
Lane A: Lonza's 50 - 1,000 bp DNA Marker (~25 ng/band)
Lane B: New England BioLabs *Msp* I digest of pBR322 (0.125 mg/lane) 20 cm long gels were run at 6 V/cm for 2 hrs. & 20 mins. Gels were post stained using Lonza's 1X GelStar® Nucleic Acid Gel Stain for 30 minutes. No destain.

Introduction

Lonza performs routine analytical testing on every lot of agarose to ensure lot-to-lot consistency for the critical characteristics that can impact functional performance. Specifications for properties such as gel strength, gelling temperature, electroendosmosis, and others are established at the optimal level for the target applications of the various agarose types. However, analytical specifications alone may not be sufficient for judging the best agarose for a specific application, nor are they the best means to compare agaroses from different vendors. The best way to find out if an agarose is right for your application, is to speak to our Scientific Support representatives, and test a sample of the recommended agarose type(s) based on your application. Lonza has extensive capabilities for modification of agarose properties to fit unique applications and requirements. Ask about our custom capabilities if one our standard products does not meet your needs.

Agarose Analytical Specifications

	Agarose	Melting Temperature	Gel Strength g/cm²	Gelling Temperature	EEO (-mr)	Moisture	Sulfate
DNA <1 kb	**NuSieve® 3:1**	≤90°C at 4%	≥1,400 at 4%	32.5°C – 38°C at 4%	≤0.13	≤10%	≤0.15%
	MetaPhor®	≤75°C at 3%	≥300 at 3%	≤35°C at 3%	≤0.05	≤10%	NA
	NuSieve® GTG®	≤65°C at 4%	≥500 at 4%	≤35°C at 4%	≤0.15	≤10%	≤0.15%
DNA >1 kb	**SeaKem® LE**	NA	≥1,200 at 1%	36°C ± 1.5°C at 1.5%	0.09 – 0.13	≤10%	≤0.15%
	SeaKem® GTG®	NA	≥1,200 at 1%	36°C ± 1.5°C at 1.5%	0.09 – 0.13	≤10%	≤0.15%
	SeaPlaque®	≤65°C at 1.5%	≥200 at 1%	26°C – 30°C at 1.5%	≤0.10	≤10%	≤0.10%
	SeaPlaque® GTG®	≤65°C at 1.5%	≥200 at 1%	26°C – 30°C at 1.5%	≤0.10	≤10%	≤0.10%
PFGE	**SeaKem® Gold**	NA	≥1,800 at 1% ≥3,500 at 1.5%	34.5°C –37.5°C at 1.5%	≤0.05	≤10%	≤0.10%
	InCert®	≤70°C at 1.5%	≥350 at 1%	26°C – 30°C at 1.5%	≤0.10	≤10%	≤0.15%
Identity Testing	**I.D.NA®**	NA	≥1,300 at 1%	36°C ± 1.5°C at 1.5%	≤0.10	≤10%	≤0.15%
Protein Electrophoresis	**SeaKem® ME**	NA	≥1,000 at 1%	36°C ± 1.5°C at 1.5%	0.16 – 0.19	≤10%	≤0.20%
	SeaKem® HE	NA	≥650 at 1%	36°C ± 1.5°C at 1.5%	0.23 – 0.26	≤10%	≤0.20%
	SeaKem® HEEO	NA	≥650 at 1%	36°C ± 1.5°C at 1.5%	≥0.30	≤10%	≤0.25%
	SeaKem® HGT	NA	≥800 at 1%	42°C ± 1.5°C at 1.5%	≤0.10	≤10%	≤0.30%
von Willenbrand's Factor Separation	**SeaKem® HGT(P)**	NA	≥1,000 at 1%	42°C ± 1.5°C at 1.5%	≤0.10	≤10%	≤0.20%
Isoelectric Focusing	**IsoGel®**	NA	≥500 at 1.5%	35°C – 45°C	Not Detectable	≤10%	≤0.20%
Cell Culture	**SeaPrep®**	≤50°C at 1%	≥75 at 2%	8°C – 17°C at 0.8%	≤0.05	≤10%	≤0.10%

Section II: Preparation of Agarose Gels

Suggested Agarose Concentrations and Dye Migration Infomation

Table 1: Suggested agaroses for particular applications

Size Range (Base Pairs)	Agarose Type	Application
20 – 800	MetaPhor® Agarose	High resolution analysis; 2% size differences
50 – 1,000	NuSieve® 3:1 Agarose	Analysis and blotting; 4% – 6% size differences resolved
	NuSieve® GTG® Agarose	Analysis and blotting; In-gel; 6% size differences resolved
1,000 – 10,000	SeaKem® GTG® Agarose	Analysis and blotting; recovery required
	SeaPlaque® GTG® Agarose	In-gel
10,000	SeaKem® Gold Agarose	Analysis

Table 2: Properties of TAE and TBE Buffer Systems

Buffer	Suggested Uses and Comments
TAE Buffer	Use when DNA is to be recovered Use for electrophoresis of large (>12 kb) DNA Low ionic strength Low buffering capacity – recirculation may be necessary for extended electrophoretic times
TBE Buffer	Use for electrophoresis of small (<1 kb) DNA Decreased DNA mobility High ionic strength High buffering capacity – no recirculation required for extended run times

Table 3: Suggested agarose concentrations for DNA sizes

	Final Agarose Concentration % (w/v)	
Size Range (Base Pairs)	1X TAE Buffer	1X TBE Buffer
SeaKem® LE and SeaKem® GTG® Agarose		
1,000 – 23,000	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
NuSieve® 3:1 Agarose		
500 – 1,000	3.0	2.0
100 – 500	4.0	3.0
10 – 100	6.0	5.0
MetaPhor® Agarose		
150 – 800	2.0	1.8
100 – 600	3.0	2.0
50 – 250	4.0	3.0
20 – 130	5.0	4.0
<80	—	5.0
SeaPlaque® and SeaPlaque® GTG® Agarose		
500 – 25,000	0.75	0.70
300 – 20,000	1.00	0.85
200 – 12,000	1.25	1.00
150 – 6,000	1.50	1.25
100 – 3,000	1.75	1.50
50 – 2,000	2.00	1.75
NuSieve® GTG® Agarose		
500 – 1,000	2.5	2.0
150 – 700	3.0	2.5
100 – 450	3.5	3.0
70 – 300	4.0	3.5
10 – 100	4.5	4.0
8 – 50	5.0	4.5
SeaKem® Gold Agarose†		
5,000 – 50,000	0.3	—
1,000 – 20,000	0.5	—
800 – 10,000	0.8	—
400 – 8,000	1.0	—

† TBE Buffer is not recommended for separation of DNA >12,000 bp.

Table 4: Migration of double-stranded DNA in relation to Bromophenol Blue (BPB) and Xylene Cyanol (XC) in agarose gels

1X TAE Buffer			1X TBE Buffer	
XC	BPB	% Agarose	XC	BPB
SeaKem® LE and SeaKem® GTG® Agarose				
24,800	2,900	0.30	19,400	2,850
11,000	1,650	0.50	12,000	1,350
10,200	1,000	0.75	9,200	720
6,100	500	1.00	4,100	400
3,560	370	1.25	2,500	260
2,800	300	1.50	1,800	200
1,800	200	1.75	1,100	110
1,300	150	2.00	850	70
NuSieve® 3:1 Agarose				
950	130	2.50	700	70
650	80	3.00	500	40
350	40	4.00	250	20
200	30	5.00	140	8
120	20	6.00	90	4
MetaPhor® Agarose				
480	70	2.00	310	40
200	40	3.00	140	35
120	35	4.00	85	30
85	30	5.00	60	15
SeaPlaque® and SeaPlaque® GTG® Agarose				
11,700	1,020	0.50	6,100	400
4,000	500	0.75	2,850	280
2,300	350	1.00	1,700	180
1,500	200	1.25	1,000	100
1,000	150	1.50	700	70
700	100	1.75	500	50
550	60	2.00	400	30
320	30	2.50	250	10
NuSieve® GTG® Agarose				
750	175	2.50	460	75
400	120	3.00	210	35
115	<20	4.00	150	<20
100	<20	5.00	80	<20
85	<20	6.00	50	<20
SeaKem® Gold Agarose				
24,800	3,550	0.30	19,000	2,550
12,200	2,050	0.50	9,200	1,500
9,200	1,050	0.75	7,100	800
6,100	760	1.00	4,000	500
4,100	600	1.25	2,550	350
2,600	400	1.50	1,900	250
2,000	330	1.75	1,400	180
1,500	250	2.00	1,000	100

Introduction

During electrophoresis, water is electrolyzed, which generates protons at the anode, and hydroxyl ions at the cathode. The cathodal end of the electrophoresis chamber then becomes basic and the anodal end acidic. The use of a buffering system is therefore required when charged molecules are electrophoresed through a separation medium. The two buffers commonly used for DNA electrophoresis are Tris-acetate with EDTA (TAE; 40 mM Tris-acetate, 1 mM EDTA) and Tris-borate with EDTA (TBE; 89 mM Tris-borate, 2 mM EDTA). Because the pH of these buffers is neutral, the phosphate backbone of DNA has a net negative charge and migrates towards the anode.

Properties of TAE and TBE Buffer Systems

Despite the apparent similarity of TAE and TBE Buffers, each has different properties which make it best suited for different applications (see table below).

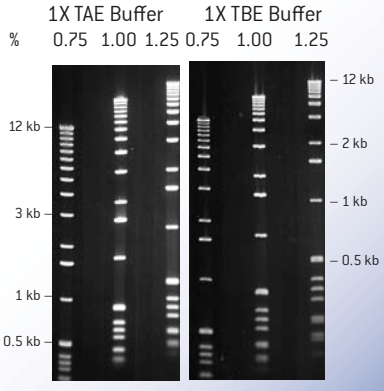
Buffer	Suggested Uses and Properties
TAE	Use when DNA is to be recovered Use for electrophoresis of large (>12 kb) DNA
	Low buffering capacity – recirculation may be necessary for extended electrophoretic times (>6 hours)
TBE	Use for electrophoresis of small (<1 kb) DNA Increased resolution of small (<1 kb) DNA
	Decreased DNA mobility High buffering capacity – no recirculation required for extended run times

Lonza offers ready-to-go buffers for electrophoresis. Refer to AccuGENE® Buffer products on page 41.

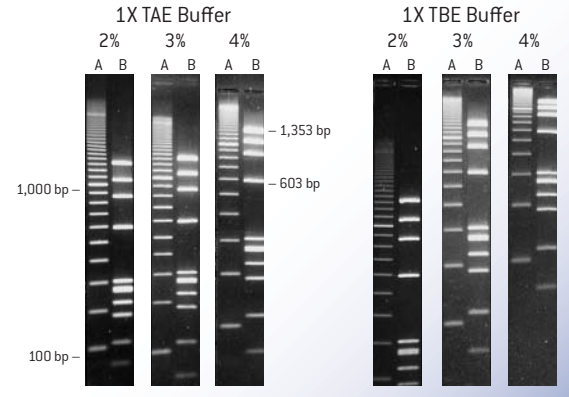
DNA Electrophoresis in TAE or TBE Buffer

When DNA will not be recovered, either 1X TAE or TBE (1X or 0.5X) Buffer is suitable for use when the DNA is less than 12 kb to 15 kb. For larger DNA, the best buffer to use for electrophoresis is TAE in combination with a low field strength (1-2 V/cm). During these extended electrophoretic runs, larger apparent gel porosity, lower EEO and low field strength decrease the tendency of large DNA to smear.

TBE Buffer is preferred for separation of small DNA (<1 kb) when DNA recovery is not required. TBE Buffer's interaction with agarose results in a smaller apparent pore size. The tighter gel reduces the broadening of DNA bands due to dispersion and diffusion.



Separation of DNA markers in 0.75% to 1.25% SeaKem® GTG® Agarose gels in 1X TAE and TBE Buffers. 1 kb DNA ladder (Invitrogen, Inc.); 1 µg/lane. The gels were cast in a 25.5 cm framing gel of 1% SeaKem® GTG® Agarose in a submarine chamber and run under 5 mm of buffer overlay at 5 V/cm for 3 hours, 30 minutes (TBE Buffer), 3 hours (TAE Buffer).

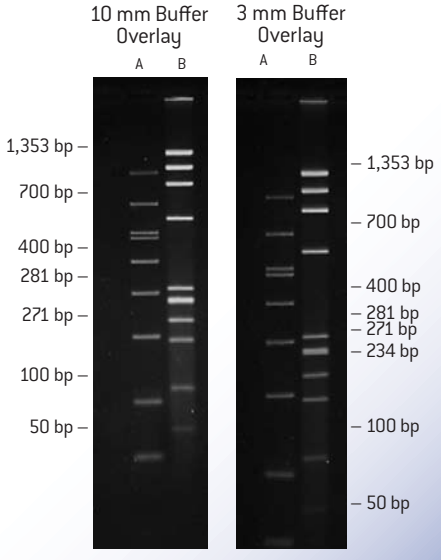


Separation of DNA fragments in 2% to 4% NuSieve® 3:1 Agarose gel in 1X TAE and TBE Buffers.
Lane A: Lonza's 100 bp Extended Range DNA Ladder (4 - 7.5 ng/band)
Lane B: Invitrogen, Inc.'s *Hae* III digest of ϕX174 (0.25 ng/band)
20 cm long gels were run at 6 V/cm for 2.5 hrs. in 1X TBE (Prepared from AccuGENE® 10X TBE Buffer). Gels were post stained using Lonza's 1X GelStar® Nucleic Acid Gel Stain for 30 minutes. No destain.

Buffer Depth

Whichever buffer is used, the depth over the gel in a horizontal electrophoretic system should be 3 mm - 5 mm. Excessive buffer depth will decrease DNA mobility, promote band distortion and can cause excessive heating within the system. Less buffer and the gel may dry out during electrophoresis.

The photograph below depicts the effect of buffer depth on DNA electrophoresis. The DNA mobility in the gel with a 10 mm buffer overlay is slower than the gel with a 3 mm buffer overlay. In the *Hae* III digested ϕX174 marker, the 281/271 bands are starting to resolve in the gel with the 3 mm buffer overlay whereas in the gel with the 10 mm buffer overlay they are not.



Separation of DNA markers in a 3% NuSieve® 3:1 Agarose gel prepared and run in 1X TBE Buffer.
Lane A: DNA marker 50 bp - 1,000 bp DNA Marker (Lonza); 0.4 µg/lane.
Lane B: *Hae* III digested ϕX174 DNA (New England Biolabs); 0.5 µg/lane.
The gels were run at 5 V/cm until the bromophenol blue tracking dye was 1 cm from the bottom of each gel.

Buffer Depletion

The rate of buffer depletion is influenced by the buffer used and its buffering capacity. Evidence of buffer depletion is gel melting, smearing of DNA and/or overheating. A 0.5X TBE Buffer has greater buffering capacity than a 1X TAE Buffer at the pH used because the pK_a of borate is closer to the initial Buffer pH than that of acetate. Standard-sized electrophoresis chambers (15 cm x 30 cm) with a 1.5 L to 2 L capacity will tolerate 40 to 50 Watt hours before buffer depletion, and buffer depletion will not occur in mini-electrophoresis chambers for 10 to 13 Watt hours. Consult the electrophoresis chamber manufacturer for specific values.

Effects of buffer depletion and development of a pH gradient can be reduced by recirculating the buffer. This is usually necessary only when electrophoresis is done for extended times or the electrophoresis buffer has a low buffering capacity.

Buffer Preparation

Alternatively, Lonza offers AccuGENE® TBE Buffer in 5X and 10X stock solutions and AccuGENE® TAE Buffer in 10X and 50X stock solutions.

50X TAE (Tris-acetate) stock

(1X=40 mM Tris base, 40 mM acetic acid, 1 mM EDTA)

242.0 g Tris base

57.1 ml Glacial acetic acid

18.61 g $Na_2EDTA \cdot 2H_2O$

To 1 liter with distilled water

5X TBE (Tris-borate) stock

(1X=89 mM Tris base, 89 mM boric acid, 2 mM EDTA)

(0.5X=45 mM Tris-borate, 1 mM EDTA)

54.0 g Tris base

27.5 g Boric acid

3.72 g $Na_2EDTA \cdot 2H_2O$

To 1 liter with distilled water

Other Buffering Systems

Tris-phosphate Buffer (TPE) may also be used for DNA electrophoresis. Like TBE Buffer, TPE has a high buffering capacity, and will not interfere with DNA recovery procedures. However, TPE can not be used when recovered DNA will be used in a phosphate-sensitive reaction.

10X Tris-phosphate Stock (TPE)

(1X=90 mM Tris base, 90 mM phosphoric acid, 2 mM EDTA)

108.0 g Tris base

15.5 ml 85% Phosphoric acid

7.44 g $Na_2EDTA \cdot 2H_2O$

To 1 liter with distilled water

Alkaline electrophoresis buffer is used for the analysis of single-stranded DNA.

Introduction

Agarose undergoes a series of steps when it is dissolved; dispersion, hydration and melting/dissolution.

Dispersion

Dispersion simply refers to the separation of the particles by the buffer without clumping. Clumping occurs when the agarose starts to dissolve before it is completely dispersed, coating itself with a gelatinous layer which inhibits the penetration of water and keeps the powder inside from dispersing. Dissolution then becomes a long process.

Hydration

Hydration is the surrounding of agarose particles by a solution (e.g., water or running buffer). Problems are sometimes encountered with hydration when using a microwave oven to dissolve agarose. In part, this occurs because hydration is time dependent and microwave ovens bring the temperature up rapidly. The problem is exacerbated by the fact that the agarose is not being agitated to help dilute the highly concentrated solution around each particle and dissolution is slowed.

Melting and Dissolution

The final stage in dissolving the agarose is the melting and dissolution. Melting can be done in either a microwave oven or on a hot plate. As the particles hydrate, they become small, highly concentrated gels. Since the melting temperature of a standard agarose gel is about 93°C, merely heating a mixture to 90°C will not completely dissolve agarose. Even low melting temperature agaroses should be boiled to ensure that all the agarose is fully dissolved. Microwave instructions for agarose preparation for gel concentrations of ≤2% w/v

Microwave Instructions for Gel Concentrations <2%w/v

1. Choose a beaker that is 2 - 4 times the volume of the solution.
2. Add room temperature 1X or 0.5X buffer and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred.
4. Remove the stir bar if not Teflon® coated.
5. Weigh the beaker and solution before heating.
6. Cover the beaker with plastic wrap.
7. Pierce a small hole in the plastic wrap for ventilation.
8. Heat the beaker in the microwave oven on HIGH power until bubbles appear.

Caution: Any Microwave solution may become superheated and foam over when agitated.

9. Remove the beaker from the microwave oven.
10. GENTLY swirl the beaker to resuspend any settled powder and gel pieces.
11. Reheat the beaker on HIGH power until the solution comes to a boil.
12. Hold at boiling point for 1 minute or until all of the particles are dissolved.
13. Remove the beaker from the microwave oven.

Caution: Use oven mits when removing beaker from microwave, as container will be hot and may cause burns.

14. GENTLY swirl the beaker to mix the agarose solution thoroughly.
15. After dissolution, add sufficient hot distilled water to obtain the initial weight.
16. Mix thoroughly.
17. Cool the solution to 60°C prior to casting.

Materials

- Microwave oven or hot plate
- Beaker that is 2 - 4 times the volume of the solution
- Teflon®-coated magnetic stir bar
- Magnetic stir plate
- Plastic wrap
- Oven mitts or other heat protection for hands

Reagents

- Distilled water
- 1X TAE, 1X TBE or 0.5X TBE Electrophoresis Buffer
- Agarose powder

Caution: Always wear eye protection, and guard yourself and others against scalding solutions.

Microwave instructions

For agarose preparation for gel concentrations ≥2% w/v

1. Choose a beaker that is 2 - 4 times the volume of the solution.
2. Add room temperature or chilled buffer (for MetaPhor® and NuSieve® GTG® Agarose) and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred to prevent the formation of clumps.
4. Remove the stir bar if not Teflon® coated.
5. Soak the agarose in the buffer for 15 minutes before heating. This reduces the tendency of the agarose solution to foam during heating.
6. Weigh the beaker and solution before heating.
7. Cover the beaker with plastic wrap.
8. Pierce a small hole in the plastic wrap for ventilation.

   For agarose concentrations >4%, the following additional steps will further help prevent the agarose solution from foaming during melting/dissolution:

   8a. Heat the beaker in the microwave oven on MEDIUM power for 1 minute.

   8b. Remove the solution from the microwave.

   8c. Allow the solution to sit on the bench for 15 minutes.
9. Heat the beaker in the microwave oven on MEDIUM power for 2 minutes.

Caution: Any microwaved solution may become superheated and foam over when agitated.

10. Remove the beaker from the microwave oven.

Caution: Use oven mits when removing beaker from microwave, as container will be hot and may cause burns.

11. GENTLY swirl to resuspend any settled powder and gel pieces.
12. Reheat the beaker on HIGH power for 1 - 2 minutes or until the solution comes to a boil.
13. Hold at the boiling point for 1 minute or until all of the particles are dissolved.
14. Remove the beaker from the microwave oven.
15. GENTLY swirl to mix the agarose solution thoroughly.
16. After dissolution, add sufficient hot distilled water to obtain the initial weight.
17. Mix thoroughly.
18. Cool the solution to 60°C prior to gel casting.

Hot plate instructions for preparing agarose

1. Choose a beaker that is 2 - 4 times the volume of the solution.
2. Add room temperature or chilled buffer (for MetaPhor® or NuSieve® GTG® Agarose) and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred to prevent the formation of clumps.
4. Weigh the beaker and solution before heating.
5. Cover the beaker with plastic wrap.
6. Pierce a small hole in the plastic wrap for ventilation.
7. Bring the solution to a boil while stirring.
8. Maintain gentle boiling until the agarose is dissolved (approximately 5 -10 minutes).
9. Add sufficient hot distilled water to obtain the initial weight.
10. Mix thoroughly.
11. Cool the solution to 60°C prior to casting.

Materials

- Microwave oven or hot plate
- Beaker that is 2 - 4 times the volume of the solution
- Teflon®-coated Magnetic Stir Bar
- Magnetic stir plate
- Plastic wrap

Reagents

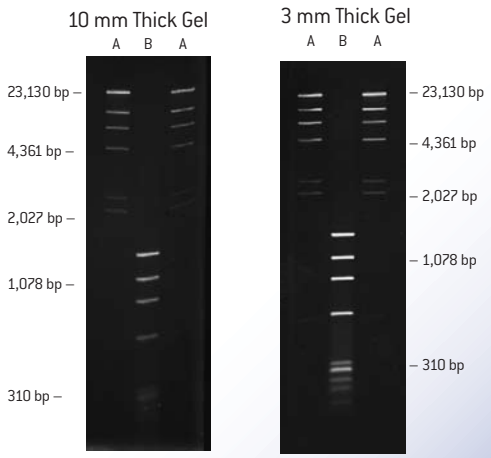
- Distilled water
- 1X TAE, 1X TBE or 0.5X TBE Electrophoresis Buffer
- Agarose powder

Caution: Always wear eye protection, and guard yourself and others against scalding solutions.

Introduction

For optimal resolution, cast horizontal gels 3 mm - 4 mm thick (see figure below). The volume of gel solution needed can be estimated by measuring the surface area of the casting chamber, then multiplying by the gel thickness. Thinner gels can be cast on GelBond® Film and/or in a vertical electrophoresis apparatus.

The photographs below depict the effect of gel depth on DNA electrophoresis. Gel thickness has a profound effect on the resolution of smaller fragments. The smaller DNA fragments in the 10 mm thick gel are fuzzy, whereas in the 3 mm thick gel the resolution is sharp throughout the gel. There is also a higher background staining in gels thicker than 5 mm.



Separation of DNA markers in 1% SeaKem® GTG® Agarose gels prepared and run in 1X TBE Buffer.
Lane A: *Hind* III digest of lambda DNA (Boehringer Mannheim); 0.1 µg/lane.
Lane B: *Hae* III digested ϕX174 DNA (New England Biolabs); 0.5 µg/lane. 20 cm long gels were run at 6 V/cm for 2 hours 15 minutes (10 mm thick gel) and 2 hours 10 minutes (3 mm thick gel).

Lonza offers a wide range of precast gels.
Refer to page 17 for information.

Horizontal gel casting instructions

1. Allow the agarose solution to cool to 60°C.
1. While the agarose solution is cooling:
   - 2a. Assemble the gel casting tray.
   - 2b. Level the casting tray prior to pouring the agarose solution.
   - 2c. Check the comb(s)* teeth for residual dried agarose. Dried agarose can be removed by scrubbing the comb teeth with a lint-free tissue soaked in hot distilled water.
   - 2d. Allow a small space (approximately 0.5 mm - 1 mm) between the bottom of the comb teeth and the casting tray.
3. Pour the agarose solution into the gel tray.
4. Replace the comb(s).
5. Allow the agarose to gel at room temperature for 30 minutes.
6. Low melting temperature agaroses and MetaPhor® Agarose require an additional 30 minutes of gelling at 4°C to obtain the best gel handling. The additional cooling step is essential for obtaining fine resolution in MetaPhor® Agarose.
7. Once the gel is set, flood with running buffer.
8. Slowly remove the comb.
9. Place the gel casting tray into the electrophoresis chamber.
10. Fill the chamber with running buffer until the buffer reaches 3 mm - 5 mm over the surface of the gel.
11. Gently flush the wells out with electrophoresis buffer using a Pasteur pipette to remove loose gel fragments prior to loading the samples.
12. Load DNA and electrophorese.

*The thickness of the comb in the direction of the electric field can affect the resolution. A thin comb (1 mm) will result in sharper DNA bands. With a thicker comb, more volume can be added to the well but the separated DNA bands may be broader.

Materials

- Horizontal electrophoresis apparatus
- Combs
- Pasteur pipette

Reagents

- Agarose solution
- Electrophoresis buffer

Vertical Gel Casting Instructions

Follow the steps below to cast a vertical agarose gel. This protocol is divided into the following segments:

— Cassette assembly

— Cassette sealing

— Casting the gel

— Preparing for electrophoresis

Cassette assembly

Unlike polyacrylamide gels, agarose gels do not adhere to glass plates and may slide out during electrophoresis. To prevent this from happening, frosted glass plates or plastic plates can be used.

Follow the steps below to assemble the glass plates.

1. Use clean glass plates. Clean with soap and water, rinse with distilled water and dry.
2. Wipe the plates with ethanol and a lint-free tissue.
3. Place two side spacers on the back plate. Follow the steps below if using glass plates.
   - 3a. For 1 mm thick standard size gels, cut a strip of Whatman® 3MM Chromatography paper (1 mm thick and 5 - 10 mm wide) long enough to fit between the two spacers.*
   - 3b. Wet with running buffer.
   - 3c. Place at the bottom of the back plate in contact with the spacers on each side (see Figure 1, page 87).
4. Put on the front plate.
5. Clamp the glass plates together.
6. Use the manufacturer's casting apparatus or seal the cassette against leaks with silicone tubing or tape.

*Alternatively, use GelBond® Film as a support for the gel, which obviates the need for the use of Whatman® 3MM Chromatography paper to hold the gel in the cassette during electrophoresis (see Chapter IX). GelBond® Film is put into the casting cassette and the gel attaches to the film during the gelling process. After electrophoresis, the gel may be dried down on the GelBond® Film and kept as a permanent record. However, GelBond® Film blocks UV light below 300 nm and exhibits background fluorescence. To overcome these problems, gels cast on GelBond® Film may be photographed inverted (gel side down) on the UV light box. Background fluorescence can be screened out by using red, orange (Wratten® #22 or #25 gelatin filter) and UV filters (Wratten® #2B gelatin filter).

Materials

- Vertical electrophoresis apparatus
- Combs and side spacers
- Whatman® 3MM Chromatography Paper
- Clamps
- Silicone tubing or electrical tape
- Two 60 ml syringes with 16-gauge needles
- Heat gun or 55°C oven
- Scalpel or razor blade

Reagents

- Agarose solution
- Electrophoresis buffer

Cassette Sealing

Follow one of the methods below to seal the cassette prior to casting the gel.

Silicone tubing method

1. Use silicone tubing which is the same diameter as the spacer thickness.
2. Cut a piece long enough to extend along the bottom and up both sides of the cassette.
3. Place the tubing across the bottom of the back plate below the blotting paper strip (see Figure 2).
4. Place the top plate over the bottom plate.
5. Clamp the glass plates together at the bottom.
6. Run the tubing up either side of the plates and finish clamping the plates together (see Figure 3).

Tape method

1. Place the top plate over the bottom plate.
2. Tape the sides of the cassette with separate pieces of tape.
3. Tape the bottom of the cassette with a separate piece of tape. This way the tape on the bottom can be removed for electrophoresis without disturbing the tape at the sides of the gel (see Figure 4).
4. Clamp the plates together.

Casting a Vertical Agarose Gel

1. Prepare agarose solution as described previously.
2. Pre-warm the assembled cassette and a 60 ml syringe for 15 minutes by placing in a 55°C oven or by using a heat gun.
3. Cool the dissolved agarose to 60°C.
4. Pour into a pre-warmed 60 ml syringe fitted with a 16-gauge needle.
5. Wedge the needle tip between the plates in the upper corner of the cassette with the needle opening directed toward the back plate (see Figure 5).
6. Inject the agarose solution at a moderate, steady rate. Keep a constant flow to prevent air bubble formation (see Figure 5).
7. Angle the cassette while pouring so the agarose solution flows down one side spacer, across the bottom and up the other side.
8. Fill until the agarose solution goes just above the glass plates.
9. Insert one end of the comb, then slowly insert the rest of the comb until the teeth are at an even depth. Insert the comb into the agarose to the minimal depth necessary to accommodate your samples (see Figure 6, page 88).

Figure 1.

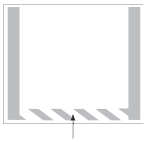


Figure 2.

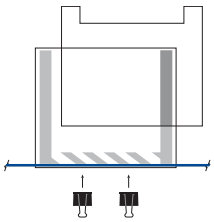


Figure 3.

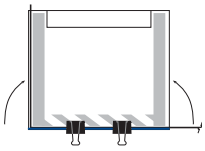


Figure 4.

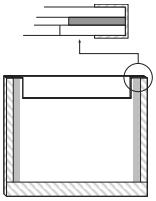
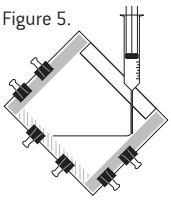


Figure 5.



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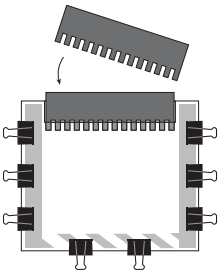
10. Place extra clamps on the side of the glass plates to hold the comb in place.
11. Cool the gel at room temperature for 15 minutes.
12. Place the gel at 4°C for 20 minutes.
13. Remove the clamps at the top of the gel.
14. Remove any excess agarose with a scalpel or razor blade.
15. Squirt running buffer in the spaces between the comb and the gel.
16. Slowly and gently lift the comb straight up. Allow air or buffer to enter the well area to release the vacuum which forms between the agarose and the comb.
17. The wells can be further cleaned by flushing with running buffer.
18. The gel can be stored overnight in a humidity chamber or in a sealed bag with a buffer-dampened paper towel.

Figure 6.



Preparing for electrophoresis

1. Remove the silicone tubing or tape at the bottom of the cassette

NOTE: If you have placed Whatman® 3MM Chromatography Paper between the plates at the bottom, it is not necessary to remove it as it will not interfere with electrophoresis.

2. Place the cassette into the chamber at an angle to minimize the number of bubbles which can collect in the well area.
3. Rinse out well area with a syringe.

NOTE: Since agarose does not adhere well to glass, leave as many clips in place as possible. For some electrophoresis chambers, it is helpful to seal the spacers at the top of the gel with molten agarose.