Agaroses

Code	Description	Molecular Weight Separation Range	Size
0710-25G 0710-100G 0710-500G	Agarose I™ General purpose	250 – 22,000 bp	25 g 100 g 500 g
K857-100TABS K857-1000TABS	Agarose I™ Tablets, 500 mg/tablet General purpose	250 – 22,000 bp	100 tabs 1000 tabs
X174-25G X174-100G X174-250G	Agarose LF™ Large Fragment Low EEO High gel strength	1000 – 40,000 bp	25 g 100 g 250 g
E776-25G E776-100G E776-250G	Agarose 3:I HRB™ High resolution blend High gel strength	50 – 2,000 bp	25 g 100 g 250 g
J234-25G J234-100G J234-250G	Agarose SFR™ Super fine resolution Low melting	20 – 2,000 bp	25 g 100 g 250 g
N605-25G N605-100G N605-250G	Agarose RA™ Routine Analysis	250 – 22,000 bp	25 g 100 g 250 g
N465-KIT	Agarose Broad Range Trial Kit Includes: Agarose I™, 3 g Agarose LF™, 3 g Agarose SFR™, 3 g Loading Buffer, 5X, 30% glycerol, 1 ml TBE Buffer Disodium, 10X Ready-Pack™* TAE Buffer, 25X Ready-Pack™**	250– 22,000 bp 1000 – 40,000 bp 20 – 2,000 bp	1 kit
N464-KIT	Agarose Small Fragment Trial Kit Includes: Agarose 3:I HRB™, 3 g Agarose I™, 3 g Agarose SFR™, 3 g Loading Buffer, 5X, 30% glycerol, 1 ml TBE Buffer Disodium, 10X Ready-Pack™* TAE Buffer, 25X Ready-Pack™**	50 – 2,000 bp 250 – 22,000 bp 20 – 2,000 bp	1 kit

*Each 10X Ready-Pack™ prepares 1L of 10X solution.
** Each 25X Ready-Pack™ prepares 1L of 25X solution.

Table I. AMRESC Agarose Application Guide

	Agarose I™	Agarose SFR™	Agarose 3:1 HRB™	Agarose LF™	Agarose RA™
All Purpose	++				++
Low Melting		++			
High Gel Strength	+		++	++	+
Low EEO				++	
Resolution of fragments < 2 kb	++	++	++	+	++
Resolution of fragments > 2 kb	++			++	++
Resolution of fragments > 40 kb				++	
High Resolution		+	++		
Super Fine Resolution		++			
PCR and Cloning	+	++			
Preparative/Sample Recovery	+	++			
Pulse-Field				++	
Southern Blotting	++		++	++	
Northern Blotting	++		++	++	
In-gel PCR		++			
In-gel Cloning		++			
In-gel Restriction Digests		++			

General Information:

AMRESCO offers a wide 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 AMRESCO agaroses provides superior clarity, maximal resolution and reproducible performance with each run. All AMRESCO agaroses are guaranteed DNase, RNase and protease free.

Advantages of agarose gel electrophoresis: The popularity of agarose gel electrophoresis, particularly for nucleic acid applications, proceeds from the ease of casting and running gels in a horizontal running apparatus known as a submarine unit. Results can be detected with a variety of easy-to-use, sensitive staining procedures. The procedure is compatible with numerous downstream applications since resolved molecules are readily recovered from gels. In addition, procedures such as PCR and restriction digests can often be performed in-gel without the need for extraction and subsequent purification. The size of nucleic acid fragments that can be resolved ranges from 20 base pair (bp) fragments to chromosomal fragments exceeding 2,000,000 bp.

AMRESCO agaroses are formulated to improve performance in a variety of specific applications. They include:

- Agarose RA™ - Routine Analysis
- Agarose I™ - General Purpose
- Agarose SFR™ - Super Fine Resolution
- Agarose 3:1 HRB™ - High Resolution Blend
- Agarose LF™ - Low EEO/High Gel Strength for Pulse Field

The appropriate agarose for a given application is dependant 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. This document focuses on electrophoresis of ds DNA between 20 and 2,000,000 bp. See Table I (page 2) for a detailed application guide to AMRESCO agaroses.

Agarose Properties: Agaroses are composed of linear polysaccharides extracted from algae. When hydrated, heated to boiling and cooled, a gel matrix is formed that can act as a sieve to resolve molecules moving through an electric field. The migration rate through the matrix is dependant on the charge to mass ratio of the molecules, the pore size of the matrix and the type of running buffer. Three critical characteristics of agaroses determine their suitability for specific applications:

Melting/Gelling Temperature: This is the temperature at which the physical state of the agarose can be reversibly changed from a solution to a gel. It can be manipulated by methylation, alkylation or hydroxylation of the polysaccharides that constitute the agarose matrix. Lower melting/gelling temperatures are an important consideration for applications requiring recovery of nucleic acids or for in-gel manipulations.

Gel Strength: The ability of an agarose gel to resist shattering during handling and downstream applications is determined by its gel strength. Agarose concentration heavily influences gel strength by affecting the degree of hydrogen bonding and double helix formation between polysaccharide molecules.

Electroendosmosis (EEO): Ions in agarose gels such as ester sulfates and pyruvates impart a net negative charge to the agarose. In the presence of an electric field these ions move towards the cathode along with associated counter-ions and water. The movement of the non-charged molecules, known as electroendosmosis, can reduce the rate of nucleic acid migration and impair band resolution during electrophoresis.

Properties affecting fragment resolution: The rate of ds DNA migration through the gel matrix is determined by both the DNA and by the pore size of the gel matrix. Pore size is determined, in turn, by the type of agarose, its concentration and the buffer in which it is dissolved.

DNA size and topology heavily influence migration rates within the gel. Migration rates for the common topological forms of ds DNA include:

- Linear, double stranded DNA - mobility is inversely proportional to the $\log_{10}$ of its molecular weight.
- Supercoiled, circular plasmid DNA - migrates faster than the equivalent molecular weight of linear DNA.
- Relaxed circular DNA (nicked circles) - migrates slower than the equivalent linearized plasmid DNA.

Table II summarizes recommended agarose concentrations and buffers for optimal resolution of a range of fragment sizes on AMRESCO agaroses.

Table I. Agarose Concentrations and Buffers for Optimal Resolution of dsDNA Fragments

Agarose	Gel %	Separation Range (bp)	Recommended Buffer
Agarose I™ • General purpose • Ideal for a variety of analytical and preparative applications	0.80	800-22,000	TAE
	1.00	500-10,000	TAE/TBE
	1.20	400-7,000	TAE/TBE
	2.00	250-5,000	TBE
Agarose RA™ • Routine analysis • Ideal for quick checks of PCR products, plasmid preps, screening and cloning	0.80	800-22,000	TAE
	1.00	500-10,000	TAE/TBE
	1.20	400-7,000	TAE/TBE
	2.00	250-5,000	TBE
Agarose LF™ • Lowest EEO ($\leq$-M_R) • High gel strength ($\geq$2000 g/cm^2) • Ideal for pulse field • Ideal for resolution of DNA fragments >20 kb	0.50	10,000-40,000	TAE
	0.70	1,000-20,000	TAE
Agarose 3:1™ HRB • High Resolution Blend • High gel strength • Ideal for small fragments and PCR products • Ideal for blotting applications	1.00	500-2,000	TBE
	2.00	250-750	TBE
	3.00	125-500	TBE
	4.00	50-250	TBE
Agarose SFR™ • Super Fine Resolution • Highest clarity • Low melting ($\leq$70ºC) • Ideal for applications requiring maximal resolution between 200 -1000 bp.	2.00	500-2,000	TAE*/TBE
	3.00	100-1,000	TAE*/TBE
	4.00	50-500	TAE*/TBE
	5.00	20-250	TAE*/TBE

* Recommended for preparative electrophoresis, although TBE buffer will offer improved resolution of the smallest bands. TAE buffer is more compatible with in-gel manipulations and band recovery procedures. DNA bands can be preserved in gels for extended periods by storing in 70% ethanol.[2]

<u>Small Fragment Resolution with AMRESCO Agaroses:</u> Small ds DNA fragments (<3 kb) are subject to broadening due to dispersion and diffusion during agarose gel electrophoresis. AMRESCO offers three types of agarose (Agarose II™, Agarose 3:I HRB™ and Agarose SFR™) specifically formulated to reduce band smearing of small fragments. Guidelines are shown in **Table III**.

Table II. Resolution of dsDNA Fragments $\leq$ 3kb

Fragment Size	Difference in Fragment Sizes Requiring Resolution	Agarose	Concentration/ Buffer	Comments
Analytical Gels				
<0.5 kb	2%	SFR™	3.5-5.0% TBE/TAE	Recommended for high clarity
<0.5 kb	3-5%	3:1 HRB™ SFR™	3.0%/TBE 3.0-4.0%/ TBE/TAE	Both exhibit high gel strength at recommended concentrations
0.5 – 2.0 kb	-	3:1 HRB™	1.5-2.5% TBE	Sharpest banding, highest gel strength for blotting
1.0-3.0 kb	-	3:1 HRB™	1.0-1.5% TBE/TAE	Sharpest banding, highest gel strength for blotting
*Preparative Gels**				
<0.5 kb	-	Agarose SFR™	1.5-2.5%/TAE	Lower gel concentrations will yield better recoveries

<u>Buffers for Agarose Electrophoresis of DNA</u> The most commonly used buffers for DNA electrophoresis are Tris-acetate with EDTA (TAE) and Tris-borate with EDTA (TBE). The neutral pH of both buffers ensures a net negative charge on DNA molecules during the run. Under these conditions the charge-to-mass ratio of the DNA fragments is constant so the rate of migration of DNA in the electric field is solely a function of fragment size.

<u>Analytical gels</u>:

- Either TAE or TBE can be used for analytical electrophoresis of DNA fragments below 15 kb.
- TBE buffer is recommended for analytical runs of fragments below 1 kb. In the presence of TBE buffer the agarose matrix forms an apparent smaller pore size that reduces the band diffusion of small DNA fragments.
- TAE is ideal for resolution of large DNA fragments since agarose gels tend to form larger apparent pore sizes in the presence of acetate ions.

<u>Preparative gels</u>:

- TAE buffer is recommended for preparative electrophoresis since some purification procedures are incompatible with TBE buffers.

Table IV below summarizes the uses of each buffer in specific applications.

Table III. Buffer Recommendations for Agarose Gel Electrophoresis

Buffer	Application/Notes
TAE	• Preparative Electrophoresis of large DNA Fragments (>12 kb) • Low buffering capacity that may require recirculation for runs longer than 6 hours
TBE	• Analytical Electrophoresis • Resolution of DNA fragments below 1 kb • Reduces DNA mobility • High buffering capacity so recirculation is not necessary for extended runs.

Storage/Stability:

AMRESCO agaroses are stable for 4 years at room temperature.

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

Protocol Overview

- In a flask 2-4 times greater than gel volume add agarose to buffer with constant swirling.
- Weigh flask
- Microwave on high for 30 seconds
- Swirl solution
- Microwave on high for 30 seconds
- Swirl solution
- Microwave on high until solution boils
- Remove and allow to cool for 1-2 minutes before swirling solution again
- Boil again for 15 seconds
- Check for crystals and repeat boiling until solution is homogenous
- Weigh flask and add water to return to original weight
- Cool to 50-55ºC
- Swirl and pour into casting stand
- Allow 30-45 minutes for gel to solidify

This overview is reprinted on page 11 of this document for easy access

Protocol:

Note: Detailed instructions are provided for agarose gel electrophoresis of ds DNA molecules. Instructions for agarose electrophoresis of ss DNA, RNA or proteins can be found in Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, 1989.

Included Reagents

Agarose

Required reagents not included:

TBE or TAE Buffer (see related product chart, page 9)
Deionized, distilled water
Sample Loading Buffer

Equipment:

Microwave or heating plate
Gel electrophoresis unit, casting stand, power supply

Caution: Microwaved solutions may become superheated and boil over or spurt when agitated or removed from the oven.

Detailed Protocol

Preparation of Agarose Solutions

Notes:

Gel formation from powdered agarose is a three phase process:

Dispersion: Agarose particles must be dispersed in buffer to avoid clumping in the subsequent hydration step. If the agarose begins to dissolve before dispersion is complete clumps of agarose become coated with a gelatinous layer which inhibits water penetration and prevents hydration of the agarose particles.

Hydration: During the hydration step, agarose particles become surrounded by buffer molecules. This step is time dependant and requires agitation to dilute the concentrated solution around each particle. Use of microwave ovens that rapidly raise the temperature of the solution in the absence of agitation can increase clumping.

Melting: Agarose must be heated to boiling to completely dissolve the agarose and achieve a homogeneous solution before gel casting.

- In general, heating times for AMRESCO agaroses are less than those for similar products from other manufacturers.
- The instructions below are for 1% agarose gels cast in horizontal (submarine) units. Higher percentage gels will require longer dispersion and hydration times.

- Vertical gels: Consult the apparatus manufacturer for casting instructions.

Microwave instructions:

Recommended for agarose concentrations ≤ 3%.

1. Agarose must be uniformly dispersed in buffer prior to hydration to avoid clumping.

   Agarose I™ tablets should be crushed into a powder with a clean rod or spatula prior to dispersion.

   Determine gel volume and agarose concentration:
   - Estimate volume:
     Volume = surface area of the casting chamber X gel depth
   - Optimal resolution is usually obtained on gels 3-4 mm thick.
2. In an appropriate container (an Erlenmeyer flask at 2-4 times the volume of the desired gel volume) slowly add agarose crystals to your buffer solution while gently swirling the flask.
3. Weigh the flask containing the buffer and agarose.
4. Heat the solution in a microwave on high power for 30 seconds. (For smaller or larger volumes, increase or decrease heating times proportionally to volume size).

   Heating times will vary depending on the wattage of your microwave oven, size of the flask used and the agarose concentration.
5. Swirl the agarose solution gently to re-suspend the particles.
6. Heat the solution another 30 seconds on high power, remove and swirl the agarose solution.
7. Place the solution back in the microwave and heat on high power until the solution just starts to boil (boiling point will probably take 10-35 seconds).

   Caution: Handle the hot flask very carefully. Microwaved solutions may become superheated and boil over when moved or touched.
8. Remove the boiling solution from the microwave oven, allow to cool briefly (1-2 minutes) at room temperature. Gently swirl the solution to release entrapped air (some air bubbles will remain).
9. Place the agarose solution back in the microwave, heat on high power and let the solution boil for approximately 15 seconds. Inspect the solution for agarose crystals (they will appear as floating "lenses") while gently swirling. If there are particles present, repeat this step until all crystals are dissolved and the solution is transparent.
10. Once the agarose is completely in solution, reweigh the flask to check for water loss by evaporation. Replenish with hot distilled, deionized water until the weight of the flask and its contents equal the original weight. Gently swirl the solution.
11. Allow the agarose solution to cool at room temperature to ~ 50-55º C before pouring the gel into the prepared casting stand. This will result in a gel with a more uniform pore size and prevent warping of the gel apparatus.

Notes: Preparation of any agarose solution in the microwave requires constant attention to prevent the solution from boiling over.
- Gently swirl agarose solution at least twice before bringing the solution to a boil.
- Once the boiling point has been reached, observe the solution after each 10-15 second boiling interval until particles are no longer visible.

Hotplate/magnetic stirrer instructions:

Particularly recommended for agarose concentrations between 4%-5%. It may be used for lower concentrations as well.

1. Follow Step 1 for microwave instructions (page 6).
2. **With heat off,** place an appropriate container (an Erlenmeyer flask at 2-4 times the volume of the desired gel volume) containing buffer and a Teflon coated stir bar on the magnetic stirrer. With rapid stirring to prevent formation of clumps slowly add agarose to your buffer solution.
3. Weigh the flask containing the buffer, agarose and stir bar.
4. With rapid stirring bring solution to a boil.
5. Maintain a gentle boil until agarose is completely dissolved, about 3-10 minutes. Inspect solution for agarose crystals and continue boiling if necessary.
6. Follow steps 9-13 in microwave instructions above (page 6).

Autoclave Instructions:

Particularly recommended for agarose concentrations between >5%. It may be used for lower concentrations as well.

Caution: Agarose solutions containing Ethidium Bromide or other mutagenic intercalating stains should not be autoclaved to avoid apparatus contamination.

1. Follow steps 1-6 for microwave instructions (page 6).

2. Cover flask with aluminum foil and autoclave at 121º C for 15 minutes.
3. Remove from autoclave and allow to cool briefly.
4. Weigh the flask and add warm distilled water to return to original weight.
5. Allow to cool to 50-55º C before pouring into casting stand.

Gel Casting

1. Assemble casting stand according to the manufacturer's instructions.
   - Casting stand should be level.
   - Comb teeth should be examined for dried agarose and cleaned with hot water and rinsed in distilled, deionized water prior to use.
2. When agarose solution cools to ~55ºC, 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 check to see that there are no air bubbles under or between the teeth of the gel comb.
4. Allow the gel to completely solidify at room temperature (about 30-45 minutes).

   Low melting agaroses and Agarose SFR™, will solidify completely at temperatures ≤ 30º C. Additional gelling time may be required.

Running Agarose Gels

1. After the gel is solidified, flood it with running buffer and carefully remove comb and dams.
2. Place in electrophoresis chamber.
3. Add running buffer until the gel is submerged 3-5 mm. Larger volumes will retard migration and generate band distortion through reduction of the voltage gradient.
4. Carefully flush well with buffer to remove loose gel fragments.
5. Sample Loading: Samples should be resuspended in 1X sample loading buffer (Code: 6X Agarose Gel Loading Dye, E190-5ML) or in buffer with an ionic strength lower than the loading buffer. If samples are resuspended in the latter, loading buffer should be added to a final concentration of 1X.

   Samples should be loaded with a pipette held **over and above** the well. Inserting the pipette into the well can cause sufficient turbulence to force the sample out the top of the well. In addition, it can tear the bottom or sides of the well so that the sample drains out of the well.

   DNA loads should not exceed 50-100 ng of DNA per band for a 3-4 mm thick gel. Overloading the DNA will reduce band sharpness.
6. Apply voltage: Attach cover and power leads and apply current to unit. Bubbles will appear around electrodes when current is flowing.

   The amount of voltage to apply is determined by the distance between electrodes, not the gel length. Band artifacts will arise if the voltage is too high or too low.
   - Voltage too high – DNA fragments over 12 kb will form distorted, streaked bands.
   - Voltage too low - DNA fragments below 1 kb will migrate slowly, resulting in fuzzy, poorly resolved bands.

   Table V summarizes optimal voltages for a range of fragment sizes and buffer types. Please consult the instructions provided by the manufacturer of the gel apparatus and power supply for further information.

Table IV. Optimal Voltages for Agarose Gel Electrophoresis

DNA Fragment Size (bp)	Voltage (V/cm)	Buffer	
		Preparative	Analytical
≤ 1 kb	5	TAE	TBE
1 kb – 12 kb	4-10	TAE	TAE/TBE
>12 kb	1-2	TAE	TAE

7. Running times: Optimal resolution and band sharpness is obtained within the upper 60% of the gel. Band sharpness may be reduced in the lower third of the gel due to broadening from diffusion and dispersion.

   Progression through the gel is usually tracked by the migration of tracking dyes included in loading buffer. The mobility of the various dyes relative to double-stranded DNA is dependant on the type and concentration of agarose and buffer in the gel. On 1% agarose, the mobility of two of the more popular dyes are:
   - Bromophenol blue – mobility equivalent to a 300 bp fragment of DNA.
   - Xylene cyanol - mobility equivalent to a 4000 bp DNA fragment.

Band Visualization

A wide variety of stains, differing in sensitivity, ease- of-use and safety considerations are available for the detection of ds DNA following electrophoresis. Stain selection is determined by the size of the DNA fragments

of interest, the stain sensitivity and compatibility with downstream applications.

Fluorescent dyes are the most commonly used stains for detection of dsDNA bands due to their sensitivity and ease of use. These dyes may be performed before, during or after electrophoresis.

- Prestains, such as AMRESCO's EZ-Vision™ DNA Dye as Loading Buffer (Code: N313-KIT), are included in the sample loading buffer. This dye binds to the DNA prior to sample loading and co-migrates with it during electrophoresis. Bands can be visualized during and immediately after the run in the absence of post-run processing.
- In-gel or pre-cast stains, such as SYBR® Green and Ethidium Bromide, are added to the agarose solution prior to casting the gel. They bind to DNA and migrate with it during the run. Some in-gel stains require post-run washing steps to reduce background staining.
- Post-run stains, such as Ethidium Bromide and SYBR® Green are commonly used to stain gels after electrophoresis. The staining procedures generally add twenty minutes to several hours of processing time before bands can be visualized.

Both fluorescent and colorimetric stains are available from a variety of manufacturers. Fluorescent stains tend to exhibit the highest sensitivity (picogram amounts of DNA) and are usually simple to use. They require illumination at specific wavelengths for excitation and may need specialized filters for gel documentation. In addition, since fluorescence is initiated or enhanced by intercalation into the DNA helix, they are often highly mutagenic. Colorimetric dyes tend to be less sensitive and require longer, more involved staining procedures.

Table VI summarizes some of the more common staining options for ds DNA agarose gel electrophoresis. Consult the manufacturers instructions to determine the stain best suited for specific applications.

Table V. Staining of ds DNA in Agarose Gels.

Stain	Type	Application	Hazard
EZ-Vision™ (Codes: N313 or N472)	Fluorescent, Prestain	ds DNA General purpose	Non-mutagenic
Ethidium Bromide (Codes: X328-1ML or E406-5ML)	Fluorescent, Precast or post stain	DNA RNA General purpose	Mutagenic
SYBR® Green	Fluorescent	DNA, RNA, High sensitivity	Mutagenic

Related Products

See the AMRESCO catalog for the entire line of products for agarose gel electrophoresis.

Product	Catalog No.
Buffers	
TBE Buffer, 5X Liquid Concentrate	J885-4L
TBE Buffer, 10X Ready-Pack™	0478-2PK
TAE Buffer, 25X Liquid Concentrate	0796-1.6L
TAE Buffer, 25X Ready-Pack™	0912-2PK
Sample Loading Buffers	
6X Agarose Gel Loading Dye, (15% Ficoll, 3 tracking dyes)	E190-5ML
Gel Loading Buffer, 4X, with Bromophenol Blue	K945-5ML
Markers	
Ready Ladder™ 100 bp	N550-300UL
Ready Ladder™ 1 kb	N551-600UL
PCR DNA Marker™ (50-2000 bp)	E854-50RXN
100 bp Ladder (100-3000 bp)	K180-250UL
1 kb Ladder (500-10,000 bp)	K181-500UL
Low-Range I™ Marker (117-14,000 bp)	E261-100UG
Low-Range II™ Marker (3500-21,000 bp)	E683-100UG
Low-Range III™ Marker (100-23,000 bp)	E687-100UG
High-Range™ Marker (10,000-48,500 bp)	E255-150UG
Wide-Range™ Marker (100-48,500 bp)	E273-200UG
Stains	
EZ-Vision™ Three (3 tracking dyes)	N313-KIT
EZ-Vision™ One™ (1 tracking dye)	N472-KIT
Ethidium Bromide Dropper Bottle	E406-5ML
Ethidium Bromide Stock Solution	X328-10ML
Destaining Bags	E732-25
Nuclease Elimination Products	
NucleasEliminator™	E891-100ML
NucleasEliminator™ Spray	E891-100ML-PUMP
NucleasEliminator™ Wipes	E891-25PK

Troubleshooting:

Clumps in gels

- *Increase agitation while dispersing agarose* – at room temperature, slowly add agarose to the buffer while swirling the flask or stirring with a stir bar.
- *Increase dispersion time* - keep solution at room temperature for 1-5 minute before beginning heating step.
- *Keep agarose dispersed during initial heating in microwave* – after 30 seconds of heating in the microwave, remove flask and swirl gently to resuspend the crystals. Repeat this step again before bringing the solution to a boil.

Bubbles in gel

- After the boiled agarose solution cools to 50-55ºC swirl gently before pouring into casting stand.

Gel overheats or melts during run

- *Excessive buffer depth* – buffer overlay should not exceed 3-5 mm.
- *Buffer depletion* – dependant on the buffer, the size of the electrophoresis chamber and the length of the run.

  Buffering capacity: 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 – buffer depletion occurs within 10 - 13 Watt hours.

  Standard chambers (1.5 to 2 liter capacity) – buffer depletion will occur in 40 – 50 Watt hours. Consult 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 the development of a pH gradient.

  Monitor pH in anode and cathode chambers during run to ensure that depletion is not occurring.

Bands are faint or invisible

- *Amount of sample loaded is too low* – increase the amount of DNA loaded. Amount loaded should not exceed 50-100 ng/band which is generally the maximum amount that can maintain sharp bands.
- *Degraded DNA* – Use nuclease free reagents, glass and plasticware during sample preparation.
- *Samples migrated off the gel*

  Increase gel concentration.

Reduce running time.
Lower voltage.

Band smearing, smiling and distortion

Smearing:

- *Sample loading volume is too large* – reduce to smallest possible volume.
- *Voltage too high* – voltage should not exceed 20 V/cm and temperature should remain < 30º C.
- *Overloaded DNA* – 50-100 ng/band is generally the maximum loadable amount that can generate sharp bands.
- *Excessive buffer depth* – buffer overlay should be 3-5 mm.
- *Buffer depletion* – See section on gel overheating.
- *Degraded DNA* – Use nuclease free reagents, glass and plasticware during sample preparation.
- *Excess salt in sample* – remove by ethanol precipitation prior to electrophoresis.
- *Protein contamination* – Phenol extract sample prior to electrophoresis.
- *Diffusion of low molecular weight bands* –
  Increase gel concentration
  Use TBE buffer for analytical applications
  Run at field strengths of 4-10 V/cm
  Switch to Agarose 3:1 HRB™ or Agarose SFR™
- *Smearing of large DNA fragments* –
  Run gel at low field strength – 1-2 V/cm
  Use Agarose LF™ with TAE buffer

Smiling:

- Band smiling can occur if the DNA begins to creep up the sides of wells prior to applying the current. Use of a loading buffer containing Ficoll® as a density agent instead of glycerol will prevent sample creep.

Gel running too slow

- *Excessive buffer depth* – buffer overlay should not exceed 3-5 mm.
- *Buffer depletion* – See section on Gel overheating.

Poor band resolution

- *Bands run too far into gel* – choose agarose and gel concentration to place fragment of interest within the top 40-60% of the gel. See Table II for recommendations.
- Use TBE buffer for fragments < 1kb for analytical electrophoresis.

References

1. Sambrook, J., et.al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, 1989.

2. Jacobs, D. and Neilan, B.A (1995) BioTechniques 19, 892.

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Protocol Overview

1. In a flask 2-4 times greater than gel volume add agarose to buffer with constant swirling.
2. Weigh flask
3. Microwave on high for 30 seconds
4. Swirl solution
5. Microwave on high for 30 seconds
6. Swirl solution
7. Microwave on high until solution boils
8. Remove and allow to cool for 1-2 minutes before swirling solution again
9. Boil again for 15 seconds
10. Check for crystals and repeat boiling until solution is homogenous
11. Weigh flask and add water to return to original weight
12. Cool to 50-55ºC
13. Swirl and pour into casting stand
14. Allow 30-45 minutes for gel to solidify

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