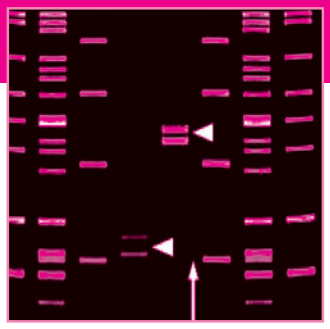
AppliCations
No.7

Improving Separation During Electrophoresis



SeparateIT gels represent a novel gel matrix for DNA electrophoresis. Gel polymers are arranged in a conceptually different way, in accordance with a new theoretical model of gel electrophoresis. SeparateIT gels selectively retard the migration of large molecules, so that DNA bands remain sharp but are more spread out relative to each other. Thanks to this increased spacing, resolving power of SeparateIT gels is at least twice higher compared to resolving power of any other gels, including polyacrylamide gels.

AppliChem

Keywords

improved resolution

Polyacrylamide Gel Electrophoresis

SeparateIT Polymer Solution

The extraordinary resolving power of SeparateIT gels results from addition of a special polymer to a polymerizing solution containing a monomer and a cross-linker. While the gels with SeparateIT-like properties could be prepared with several different polymers, chemical composition and molecular weight of the polymer required a careful optimization for each particular monomer/cross-linker combination.

In order to satisfy numerous requests from researchers who wish to improve resolving power of their acrylamide based gels, AppliChem now provides SeparateIT Polymer Solution. This polymer has been optimized for the gels with a ratio of acrylamide to N,N-methylene-bisacrylamide of 29 : 1. The polymer is not optimized for denaturing, urea-containing polyacrylamide gels.

SeparateIT Polymer Solution comes as a 10X solution. It should be mixed with a buffered solution of acrylamide and Bis prior to addition of TEMED and ammonium persulfate. Gels with SeparateIT Polymer Solution are prepared in the same way as any regular polyacrylamide gels. Likewise, the electrophoresis, gel staining and recording are carried out as usual. The increased resolving power of SeparateIT gels enables full separation of closely spaced bands on short gels. For example, a pair of fragments differing by 4 bp is usually resolved on less than 4 cm of gel length. Two DNA fragments in the 70–150 bp range that differ by 1 bp can be separated on SeparateIT gels that are 8 cm long.

We recommend that Mini gel cassettes, which are 8 or 10 cm long, are used for casting acrylamide-Bis gels with SeparateIT Polymer Solution. Such relatively short gels will be appropriate for the majority of demanding separations which have previously required 20–30 cm long polyacrylamide gels. The use of shorter gels is beneficial for several reasons, including easier gel preparation and handling, lower cost of the gel materials, faster electrophoresis runs, and lower consumption of gel staining reagents. In addition, a lower amount of sample DNA needs to be loaded on a gel with a high resolving power compared to a gel with a low resolving power. This is the case because DNA bands that migrate a short distance remain sharper than the bands that migrate a long distance. It is always advantageous when closely spaced bands separate after migrating just a few centimetres.

Protocol for preparing acrylamide-Bis gels with SeparateIT Polymer Solution

Typical 1 mm thick 10 cm long Mini gels have a volume of about 10 ml. Thinner or shorter gels require only about 6 ml of gel solution. The amounts of reagents for 10 ml gels are provided in Table 1 and for 6 ml gels in Table 2.

After adding TEMED and ammonium persulfate, the polymerizing solution is quickly pipetted into a glass cassette, which is filled to about 1 cm below edge of the shorter glass plate. The gel solution is immediately overlaid with about 150 µl of 50 % methanol in water that is carefully pipetted into the corners of the cassette. The gel will polymerize in about 10 min. The appearance of the gel is slightly opaque. After additional 5 min, remove the overlaying solution and any unpolymerized gel solution.

When sample wells are formed directly in the separating gel, whose concentration is usually from 6 to 12 %, then DNA bands are often irregular because of uneven gel polymerization at the interface with the comb, especially when the comb is made of teflon. This problem is avoided by forming sample wells in another, low percentage gel. That gel is similar to a stacking gel in discontinuous buffer SDS protein electrophoresis, but we cannot call it a stacking gel because the buffer system is continuous here. The composition of this 4 % gel is as follows:

Acrylamide-Bis 40 % (29 : 1) Stock Solution	1.0 ml
Buffer Stock Solution (1.2 M TAE, 10X)	1.0 ml
Deionized Water	8.0 ml
TEMED	5 µl
Ammonium persulfate (10 %)	50 µl

Before adding ammonium persulfate, use about 0.5 ml of the above solution for rinsing the gel surface. The polymerizing solution is pipetted in the remaining space above the separating gel, and the comb is inserted carefully to prevent entrapment of air bubbles. The gel should polymerize in 10–12 min. Take the gel cassette in your hand and position it horizontally, add a few drops of running buffer on the comb, and carefully pull out the comb. Even if some sample wells are not perfect, any initial unevenness in band shape will be corrected at the time when the band reaches the top of the separating gel.

Loading buffer

Prepare a stock solution of a loading buffer containing about 50 % sucrose, or glycerol, in 150 mM TAE buffer containing Xylene cyanol. Mix 3–4 parts of your sample with 1 part of the loading buffer.

Electrophoresis buffer

The running buffer is 60 mM TAE (10X stock solution is available from AppliChem Product No. A1416). Other buffers can be used for the gel buffer and running buffer in place of 60 mM TAE, but some optimizations may be necessary. We have obtained inferior results with the regular TBE buffer compared to 60 mM TAE buffer. Therefore, don't use TBE buffer.

Electrophoresis conditions

After inserting the cassette into the electrophoresis apparatus, rinse the wells with the running buffer. Load samples to the bottom of the sample wells.

Electrophoresis is typically conducted for 80–120 min at 200 V when the gel cassette is 8 cm long, corresponding to an electric field strength of 25 V/cm. As a rule of thumb, run a gel at least till Xylene cyanol reaches the gel end. Optimal running time will depend on the DNA samples being analyzed. For separating DNA fragments that differ by 1–3 bp, the gel concentration and running time should be chosen such that the two closely spaced bands migrate near the gel bottom. The running time depends also on design of the electrophoresis apparatus, as heat capacity differs from one apparatus to another. Therefore, even at an equal electric field strength, the actual gel temperature, and accordingly DNA migration rate, may vary considerably from one apparatus to another. In the instruments that have a low buffer volume in the cathode compartment, temperature of the running buffer in that compartment may rise to 50–55°C at the end of the run. This high temperature does not affect quality of the separation.

In electrophoresis units that feature temperature control, we recommend that the gels are run at 55°C in order to shorten the running time and to reduce sequence-dependent anomalous mobility of DNA fragments.

Table 1. Gel volume of 10 ml.

Gel Concentration	DNA Range bp	Acrylamide-Bis, 40 %, (29 : 1)	Deionized Water	TAE Buffer (10X)	SeparateIT Polymer Solution (10X)	TEMED	Ammonium persulfate
12 %	50–150	3.00 ml	5.00 ml	1.00 ml	1.00 ml	5 µl	50 µl
11 %	60–200	2.75 ml	5.25 ml	1.00 ml	1.00 ml	5 µl	50 µl
10 %	70–250	2.50 ml	5.50 ml	1.00 ml	1.00 ml	5 µl	50 µl
9 %	90–300	2.25 ml	5.75 ml	1.00 ml	1.00 ml	5 µl	50 µl
8 %	100–350	2.00 ml	6.00 ml	1.00 ml	1.00 ml	5 µl	50 µl
7 %	120–400	1.75 ml	6.25 ml	1.00 ml	1.00 ml	5 µl	50 µl
6 %	150–800	1.50 ml	6.50 ml	1.00 ml	1.00 ml	5 µl	50 µl

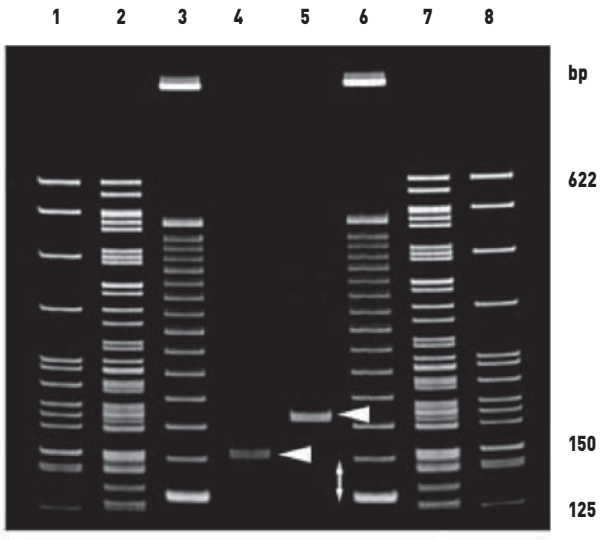
Table 2. Gel volume of 6 ml.

Gel Concentration	DNA Range bp	Acrylamide-Bis, 40 %, (29 : 1)	Deionized Water	TAE Buffer (10X)	SeparateIT Polymer Solution (10X)	TEMED	Ammonium persulfate
12 %	50–150	1.80 ml	3.00 ml	0.6 ml	0.6 ml	3 µl	30 µl
11 %	60–200	1.65 ml	3.15 ml	0.6 ml	0.6 ml	3 µl	30 µl
10 %	70–250	1.50 ml	3.30 ml	0.6 ml	0.6 ml	3 µl	30 µl
9 %	90–300	1.35 ml	3.45 ml	0.6 ml	0.6 ml	3 µl	30 µl
8 %	100–350	1.20 ml	3.60 ml	0.6 ml	0.6 ml	3 µl	30 µl
7 %	120–400	1.05 ml	3.75 ml	0.6 ml	0.6 ml	3 µl	30 µl
6 %	150–800	0.90 ml	3.90 ml	0.6 ml	0.6 ml	3 µl	30 µl

Staining

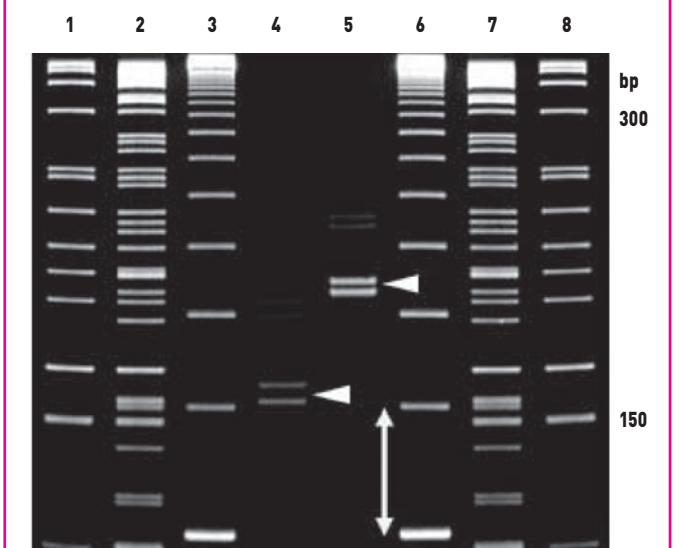
The gels with SeparateIT Polymer Solution can be stained with all stains commonly used for detecting nucleic acids in polyacrylamide gels. Staining time of 20 min is sufficient when using ethidium bromide (0.5 µg/ml) or SYBR® dyes. For best sensitivity, 254 nm UV light should be used for gel recording. The gels can also be silver stained. Typical results are shown in Figures 1–3.

Figure 1 BEFORE



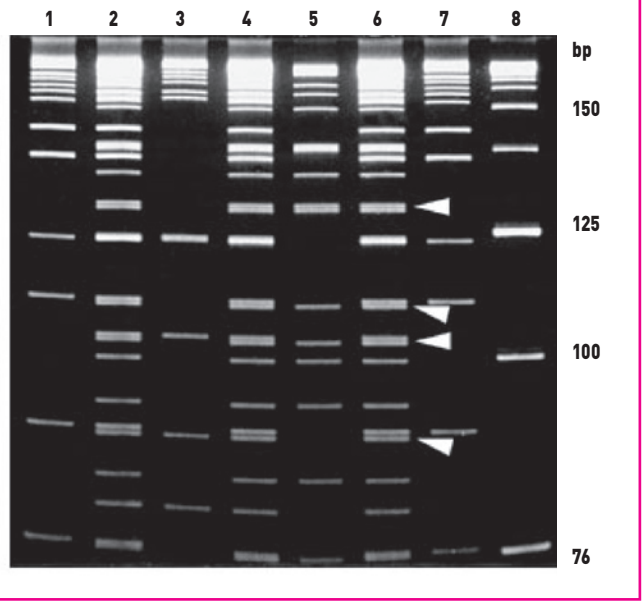
A vertical 8 cm long 8 % Acrylamide-Bis (29 : 1) gel was run at 200 V for 70 min. Lanes: 1,8 – pBR322/Msp I; 2,7 – M3 Marker; 3,6 – 25 bp ladder, 4 – vWA alleles 16 and 17 (151 and 155 bp); 5 – THO1 alleles 6 and 7 (183 and 187 bp). These microsatellite bands are not resolved.

Figure 2 NOW



A vertical 8 cm long 8 % Acrylamide-Bis (29 : 1) gel containing 1X SeparateIT Polymer Solution was run at 200 V at identical conditions. Samples are identical to those loaded to the gel on the left. In the 25 bp ladder, the spacing between the 125 bp and 150 bp fragments increased 2.8 fold. The 151/155 and 183/187 bp alleles are fully resolved.

Figure 3



A vertical 8 cm long 11 % Acrylamide-Bis (29 : 1) gel containing 1X SeparateIT Polymer Solution was run at 200 V for 90 min. Lanes: 1,7 - pBR322/Msp I; 2, 4, 6 – M3 Marker; 3 – pBR322/Hae III, 7 – pBR322/Hha I, 8 – 25 bp ladder. The marked band pairs include 89/90, 103/104, 109/110 and 131/132 bp.

Fields of AppliCation

- Analytical gels
- Genotyping
- Diagnostics
- Food testing
- GMO control
- SSPE
- Microsatellite analysis

Troubleshooting

Problem	Cause	Action
Gel does not polymerize, or it polymerizes after more than 15 min	Old ammonium persulfate	Make a fresh solution
	Old TEMED	Purchase a new one
	Too low gel concentration	Take a 4 % solution at least
	Acrylamide of poor quality	Take electrophoresis grade
Uneven top surface of the separating gel	Gel solution not overlaid	Overlay it with 50 % methanol
	Overlaid with water	Use 50 % methanol in water
	Overlaying solution mixed with gel solution	Carefully add the overlaying solution
	The solution left for over 30 min	Remove the solution earlier and rinse the gel surface
Irregular, distorted or diffuse bands	Uneven top surface	Overlay it with 50 % methanol
	Sample wells formed in separating gel	Polymerize a second 4 % gel on top of the separating gel
	High salt amount in samples	Dilute or dialize the samples
	Incorrect buffer	Use 60 mM TAE as the gel and running buffer
	Poor gel polymerization	Change monomer solution, TEMED and persulfate
Expected resolution is not achieved	Incorrect ratio acrylamide : Bis	Use only 29 : 1
	Incorrect gel concentration	See Table 1
	Running time too short	Let the bands migrate 4 cm at least
	Gel overloaded	Load 1/10 of DNA amount
Particles visible in the gel	Dust particles on glass plates	Clean the plates
	Particles in gel solution	Filter polymerizing solution through a 0.45 µm disc attached to a syringe
High background	Gel not destained	Destain the gel
	Old ethidium bromide	Prepare a fresh solution
	SYBR® Green exposed to light	Perform staining in the dark
	Incorrect photography filters	Check the filters
	Illumination at 312 nm	Switch to 254 nm
	Staining without shaking	Place the tray on a shaker
Runs are not reproducible	No temperature control	Use an apparatus with buffer circulation
	Depletion of buffer ions	Use an apparatus with temperature control

SeparateIT Polymer Solution

Product No.	Quantity
A8587,0050	50 ml
A8587,0100	100 ml
A8587,0500	500 ml
A8587,1000	1 L

Gel Electrophoresis Size Marker

Our products on offer includes *ready-to-use* DNA and protein size marker, as well as lyophilized DNA size marker, overcoming the problem of stability.
For more information about size marker please see our brochure.

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