Technical Manual

P/N 3551 EnhanceIT Polymer Solution 50 ml
P/N 3552 EnhanceIT Polymer Solution 250 ml
P/N 3553 EnhanceIT Polymer Solution 500 ml
P/N 3554 EnhanceIT Polymer Solution 1000 ml

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1. Important Information

Handle EnhanceIT Polymer Solution and gels containing the solution with care! Ware protective clothing, gloves and safety goggles.

1. 1 Specifications

EnhanceIT Polymer Solution is intended for Research Use Only!

- EnhanceIT Polymer Solution should be stored at 4 °C (40 °F).
- Shelf-life: up to 12 months at 4 °C (40 °F).
- EnhanceIT Polymer Solution comes as a 10 x stock solution (v/v).
- EnhanceIT Polymer Solution has been optimised for DNA gels with a ratio of acrylamide : N,N-methylene-Bis-acrylamide of 29 : 1.
- EnhanceIT Polymer Solution is not suitable for denaturing polyacrylamide gels containing urea.

1. 2 General remarks

Before getting started, please read the following instructions carefully.
The technical manual will provide detailed instructions to prepare polyacrylamide gels containing EnhanceIT Polymer Solution for high-resolution gel electrophoresis of double stranded DNA. It also instructs on general electrophoresis conditions and staining procedures.

abc biopply reserves the right to make changes to the following instructions without notification. For the latest version of the technical manual, please refer to our web page: www.biopply.com

2. Introduction

Gels containing EnhanceIT Polymer Solution consist of a novel gel matrix in which the polymers are arranged in a conceptually different way based on an alternative theoretical model for gel electrophoresis [1]. EnhanceIT Polymer Solution thereby specifically retards the migration of larger DNA molecules. Consequently, differently sized fragments are resolved better, yet the DNA bands remain sharp and focussed. The resolution of gels containing EnhanceIT is therefore up to 3 times higher than any other gel.

EnhanceIT Polymer Solution has been optimised for polyacrylamide gels with a ratio of 29 : 1 (acrylamide : N,N-methylene-Bis-acrylamide). The solution is not suited for denaturing gels containing urea.

Polyacrylamide gels containing EnhanceIT Polymer Solution are prepared and handled like standard polyacrylamide gels. That applies to protocols to run the electrophoresis, stain the gels and record the results.
The increased resolving power of EnhanceIT-containing gels, however, enables complete separation of bands on relatively short running distances, hence shorter gels. For example: two bands differing by 4 bp can usually be resolved in less than 4 cm. Similarly, two fragments in the range of 70 to 150 bp that differ by 1 bp only can be separated on 8 cm gels.
We recommend to use mini gel cassettes of 8 to 10 cm length for gel casting. These will be sufficient for the majority of high-resolution separations that on standard acrylamide gels required 20 to 30 cm running length.
The reduction in gel size is beneficial for several reasons:

- easier gel preparation and handling
- lower costs of chemicals
- faster electrophoresis runs
- reduced volumes of gel staining reagents

Furthermore, EnhanceIT-containing gels are more sensitive than standard polyacrylamide gels since the increased resolving power allows for shorter migration distances and consequently sharper, hence more compact, DNA bands resulting in intensified signals.

EnhanceIT Polymer Solution is provided as a 10 x stock solution (v/v). When preparing the acrylamide polymerisation mix, this stock solution should be mixed with buffered acrylamide and Bis ***prior*** to the addition of TEMED and ammonium persulfate!

[1] Anal. Biochem. (1995) 231: 1-12

3. Protocol

3. 1 Preparation of acrylamide-Bis gels containing EnhanceIT Polymer Solution

Mini gel cassettes are normally 10 cm long. Depending on their thickness (1 mm or less), they contain a volume of either about 10 ml (Table 1) or 6 ml (Table 2).

Table 1: Gel volume of 10 ml

Gel Conc. [in %]	DNA Range [in bp]	Acrylamide-Bis, 40%, (29 : 1) [in ml]	Deionized Water [in ml]	TAE Buffer (40x) [in ml]	EnhanceIT Polymer Solution [in ml]	TEMED [in µl]	Ammonnium Persulfate (10%) [in µl]
12	50-150	3.00	5.50	0.5	1.00	5.0	50
11	60-200	2.75	5.75	0.5	1.00	5.0	50
10	70-250	2.50	6.00	0.5	1.00	5.0	50
9	90-300	2.25	6.25	0.5	1.00	5.0	50
8	100-350	2.00	6.50	0.5	1.00	5.0	50
7	120-400	1.75	6.75	0.5	1.00	5.0	50
6	150-800	1.50	7.00	0.5	1.00	5.0	50

Table 2: Gel volume of 6 ml

Gel Conc. [in %]	DNA Range [in bp]	Acrylamide-Bis, 40%, (29 : 1) [in ml]	Deionized Water [in ml]	TAE Buffer (40x) [in ml]	EnhanceIT Polymer Solution [in ml]	TEMED [in µl]	Ammonnium Persulfate (10%) [in µl]
12	50-150	1.80	3.30	0.3	0.6	3.0	30
11	60-200	1.65	3.45	0.3	0.6	3.0	30
10	70-250	1.50	3.60	0.3	0.6	3.0	30
9	90-300	1.35	3.75	0.3	0.6	3.0	30
8	100-350	1.20	3.90	0.3	0.6	3.0	30
7	120-400	1.05	4.05	0.3	0.6	3.0	30
6	150-800	0.90	4.20	0.3	0.6	3.0	30

- After the addition of TEMED and ammonium persulfate, the solution is quickly filled into the corresponding casting cassette. Fill the cassette to about 1 cm below the edge of the shorter plate.
- Overlay the gel solution immediately with approximately 150 µl of 50% (in ddH_2O) isopropanol (or ethanol).
- Polymerisation will take about 10 minutes. When polymerised, the gel matrix turns slightly opaque. After additional 5 minutes, remove the overlaying isopropanol and any unpolymerised gel solution.

Stacking gel for sample slots:
We recommend to subsequently overlay the separation gel with a 4% stacking gel similar to SDS protein electrophoresis (Note: in contrast to protein electrophoresis, there is not discontinuous buffer system with EnhanceIT-containing DNA gels). The stacking gel will serve to form the sample slots.

Acrylamide-Bis, 40%, (29 : 1) [in ml]	Deionized Water [in ml]	TAE Buffer (40x) [in ml]	TEMED [in µl]	Ammonnium Persulfate (10%) [in µl]
1.0	8.5	0.5	5	50

- Before adding the ammonium persulfate, use about 0.5 ml of the stacking solution to rinse the previously prepared separation gel.
- Add the ammonium persulfate to the stacking solution and quickly fill up the casting cassette.
- Carefully insert the comb to avoid the formation of air bubbles.
- Polymerisation takes about 10 to 12 minutes.

3. 2 Loading buffer

We recommend to use 5 x concentrated loading buffer from abc biopply (P/N 3033 for 10 x 1 ml: or 3034 for 50 x 1 ml).
Alternatively, a loading buffer containing 50% sucrose or glycerol in 150 mM TAE buffer can be prepared. For visualisation, use bromophenol blue.

3. 3 Electrophoresis running buffer

We recommend to use 40 x concentrated TAE running buffer from abc biopply (P/N 3031). Alternatively, prepare a 40 x TAE stock solution including the following ingredients:

Tris (hydroxymethyl) aminnomethane [in g]	$Na_2EDTA• 2H_2O$ [in g]	Acetic Acid (glacial) [in ml]
145.37	11.16	34.4

- Dissolve Tris and Na_2EDTA in 800 ml of ddH_2O.
- Add the acetic acid in a fume hood and adjust the volume to 1 litre with ddH_2O.

We do not recommend to use borate containing buffers (such as TBE) since they lead to inferior results.

3. 4 Gel electrophoresis

Before loading DNA samples, it is recommendable to rinse all sample wells with running buffer. The samples are then loaded to the bottom of each well.

Generally, we recommend to run the gels at approximately 25 V/cm. For an 8 cm gel cassette this equals to 200 V. Under those conditions, typical running times are around 80 to 120 minute. However, the optimal running time may vary depending on the required resolution and the DNA fragment lengths. To achieve a 1 to 3 bp resolution, ideally the two fragments should be run about ⅔ of the maximal running distance and the gel. The concentration and running time need to be optimised accordingly.

The running time also depends on the geometric design of the electrophoresis apparatus. Similarly, heat generation during the run may vary considerably between different units and alter DNA migration and running time, even when the run was performed at the same electric field strength.

In some units, the temperature during the run get as high as 50 to 55°C. However, higher temperature does not effect separation. On the contrary, for units featuring temperature control, we do recommend to run the electrophoresis at 55°C to reduce running time but also to eliminate sequence-dependent migration artefacts.

3. 5 Staining

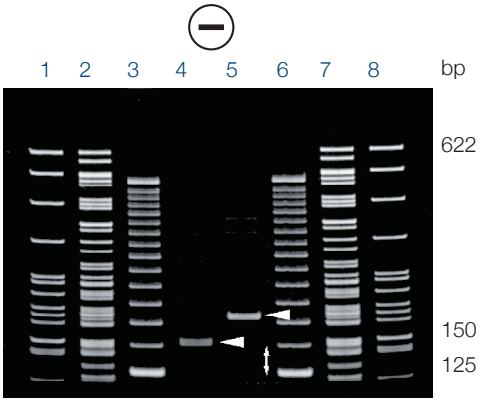
EnhanceIT-containing polyacrylamide gels can be stained with all dyes commonly used for DNA detection in polyacrylamide gels.
For ethidium bromide or SYBR dyes, we recommend to stain the gels for approximately 20 minutes (254 nm excitation).
Alternatively, the gels can also be stained using silver stain protocols.

Comparisons of polyacrylamide gels with or without EnhanceIT Polymer Solution are shown in figures 1 to 3.

4. Results

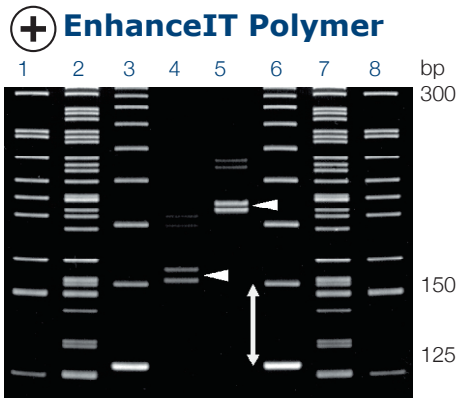
4. 1 Comparison of polyacrylamide gels with or without EnhanceIT Polymer Solution

Figure 1:



A vertical 8 cm long 8% acrylamide-Bis (29 : 1) gel was run at 200 V for 70 minutes.
Lanes:
1, 8 - M1 Marker (abc biopply); 2, 7 - M3 Marker (abc biopply); 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).
The indicated microsatellite bands are not resolved!

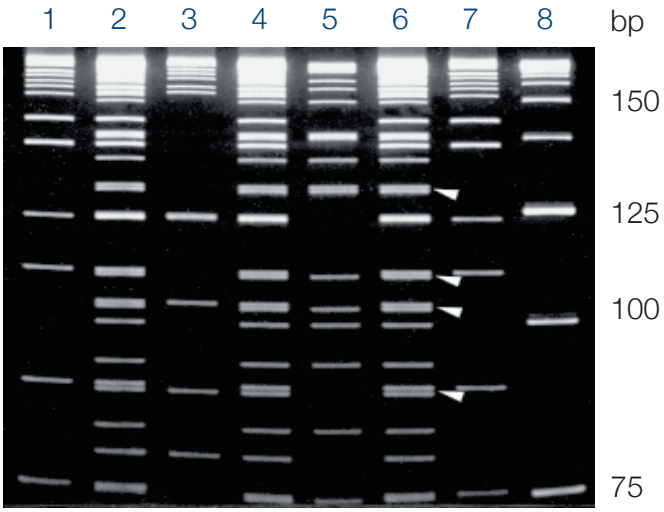
Figure 2:



A vertical 8 cm long 8% acrylamide-Bis (29 : 1) gel containing 1 x **EnhanceIT Polymer Solution** was run equally to the gel on the left.
Sample order identical to left side panel.
Lane 6: the spacing between the 125 bp and 150 bp fragments is increased by about 3-fold. Lane 4, 5: the 151/155 bp and 183/ 187 bp alleles indicated by an arrow are fully resolved.

4. 2 Resolve 1 bp differences on a mini gel with EnhanceIT Polymer Solution

Figure 3:



A vertical 8 cm long 11 % acrylamide-Bis (29 : 1) gel containing 1 x **EnhanceIT Polymer Solution** was run at 200 V for 90 minutes.
Lanes1, 7 - M1 Marker (abc biopply); 2, 4, 6 - M3 Marker (abc biopply);
3 - pBR322/HaeIII; 7 - pBR322/HhaI; 8 - 25 bp ladder.
Lane 6: arrowheads indicate resolved band pairs 89/90, 103/104, 109/110 and 131/132 bp.

5. Troubleshooting

Trouble	Shoot
Gel polymerises very slowly (> 15 minutes) or not at all.	- Replace the ammonium persulfate solution. - Replace TEMED. - The acrylamide concentration is too low (< 4%). - Use electrophoresis grade acrylamide.
The upper surface of the separation gel looks irregular/uneven.	- Make sure the separation gel is overlaid immediately after pouring with a solution of 50% isopropanol or ethanol. - Be careful that the isopropanol or ethanol solutions do NOT mix with the polymerisation solution. - The isopropanol or ethanol solution has been left on top of the separation gel for too long. Remove the overlay after maximal 30 minutes.
Irregular, distorted or diffuse DNA bands.	- Uneven surface of separation gel (see above). - Make sure a stacking gel is used for the sample wells. - Very high salt concentration in DNA samples may interfere with DNA migration. Dilute or dialyse the sample. - Always use 30 mM TAE running buffer. Potentially, use higher salt concentration. - Polymerisation was poor: change monomer solution, TEMED and ammonium persulfate solution.

Trouble	Shoot
The observed resolution is below expectation.	- Make sure the ratio of acrylamide : N,N-methylene-Bis-acrylamide is 29 : 1. - Ensure to use the proper gel concentration (see Tables 1 and 2). - Make sure that the bands of interest have migrated a distance of at least 4 cm. - Extended DNA concentration my temper with resolution. Dilute the sample accordingly.
Solid particles are visible in the gel matrix.	- Ensure to work with properly clean glass plates only. - Filter the polymerising solution (pore size: 0.45 µm).
High background after staining.	- destain the gel in ddH_2O after staining. - Ensure to use fresh ethidium bromide solution only. - SYBR dyes are sensitive to light exposure. Therefore make sure to stain in a dark environment only. - Make sure to use the appropriate filters for the chosen dye. - Only excite at 254 nm (312 nm causes autofluorescence of the gel matrix). - Best staining is performed on a shaker/ rocking platform.

6. Related Products

Product Number	Product
3031	40 x Running buffer Sock solution, 1.2 M TAE buffer
3033	Sample Loading Buffer, 10 ml
3034	Sample Loading Buffer, 50 ml
3035	Sample Loading Buffer, 2 dyes, 10 ml
3036	Sample Loading Buffer, 2 dyes, 50 ml
3203	M3 DNA size marker
3204	M1 DNA size marker

For further questions, please visit our web page or contact us.