SDS-PAGE

Separation Technique File No. 110

PhastSystem™

This technique file describes optimized methods for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with PhastGel® gradient 10–15 and PhastGel gradient 8–25 using PhastGel SDS buffer strips. SDS-PAGE with PhastGel gradient 4–15 is described in Separation Technique File No. 130.The methods have been optimized using crude protein extracts and commercially available proteins. Therefore, they are generally applicable and offer a good starting point for developing methods for specirfic applications.

This file gives only specific method information. Detailed descriptions of how to program separation methods, how to load sample applicators, and how to run PhastGel gradient media are given in the Users Manual of PhastSystem.

Introduction

SDS-PAGE with discontinous buffer systems is one of the most common electrophoresis methods in use today, and is widely used for molecular weight (MW) measurements and analysis of polypeptides (1,2). Discontinuous buffer systems in combination with polyacrylamide (PAA) gradient gels enhance resolution and band sharpness.

To these advantages, PhastSystem adds speed, reproducibility and convenience; separations take approximately 30 minutes, run under exact, programmed conditions, and require no buffer preparation.

Instructions for MW measurements using PhastGel gradient media and the Pharmacia MW calibration kit proteins are given in the Users Manual of PhastSystem.

PhastGel gradient media

PhastGel gradient media for SDS-PAGE comprises three PAA gels, PhastGel gradient 10–15, PhastGel gradient 8–25 and PhastGel gradient 4–15, and PhastGel SDS buffer strips.

All gels have a 13 mm stacking gel zone (4,5% T, 3% C) and a 32 mm gradient gel zone. PhastGel gradient 10–15 has a continuous 10 to 15% gradient gel zone with 2% crosslinking. PhastGel gradient 8–25 has a continuous 8 to 25% gradient gel zone with 2% crosslinking and PhastGel gradient 4–15 has a continuous 5–15% gradient gel zone with a continuous 1—2% gradient crosslinking. The gels are approximately 0.45 mm thick.

The buffer system in the gels is of 0.112 M acetate (leading ion) and 0.112 M Tris, pH 6.4.

The buffer system in PhastGel SDS buffer strips is of 0.20 M tricine (trailing ion), 0.20 M Tris and 0.55% SDS (analytical grade), pH 8.1. The buffer strips are made of 3% Agarose IEF.

Molecular weight range

The gradient in the gradient gel zone determines the molecular weight (MW) range that is possible to analyze in one run. PhastGel gradient 10–15 is designed to give a linear relationship between a protein's migration distance and the logarithm of its MW for the MW range 10 000 to 250 000 for SDS-denatured proteins. Proteins above 250 000 are too large to enter the gradient gel. Proteins below 10 000 may appear on the developed gel, but their migration distance may not be in linear proportion to log MW.

Similarly, PhastGel gradient 8–25 gives a linear relationship between a protein's migration distance and log MW for the MW range 6 000 to 300 000 for SDS-denatured proteins.

Principle of the method

Proteins are first dissociated into their polypeptide subunits by SDS and β-mercaptoethanol. The subunits bind the same amount of SDS per unit weight (1.4 g SDS per gram of protein (3)) and take on a net negative charge. All polypeptides will have

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the same charge density; therefore, separation will depend largely on their size. Bromophenol blue is usually added to the samples as a tracking dye.

When electrophoresis starts, the leading and trailing ions (acetate/tricine) form a moving boundary that starts to migrate through the stacking gel. The mobility of the proteins in the stacking zone is relatively unaffected by the gel; proteins will stack between the leading and trailing ions. SDS migrates from the cathode buffer strip at a low concentration and stacks at the front of the moving boundary along with the bromophenol blue.

The moving boundary is a front between regions of high and low voltage. The pH behind the boundary is approximately 8.5. The propagation of the voltage gradient or the moving boundary through the gel is illustrated in figure 1.

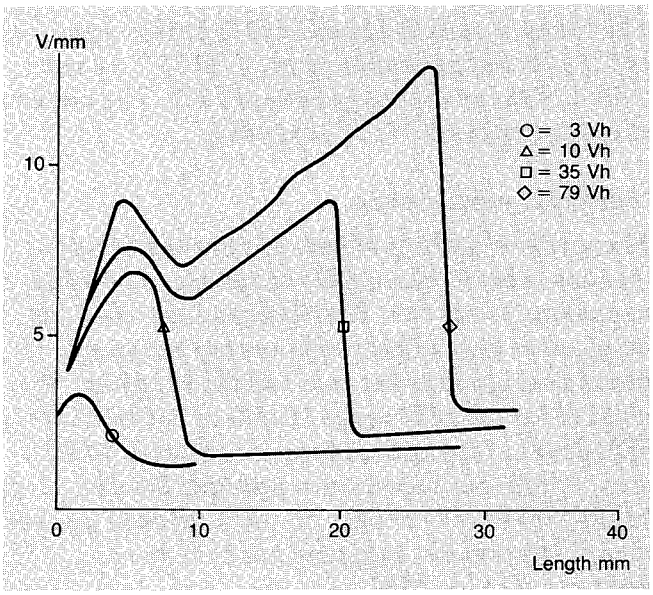


Fig. 1. The propagation of the voltage gradient through the gel. Measurements were taken after 3, 10, 35 and 79 Vh.

When the proteins reach the stacking/gradient gel interface, their mobility is drastically reduced, due to the sudden decrease in pore size, and the moving boundary rapidly migrates away from the proteins. The proteins unstack and migrate at a uniform voltage and at constant pH (pH 8.5). As the proteins migrate through the gradient gel they separate according to their size.

The gradient sharpens the protein bands, since the advancing edge of the protein is retarded more than the trailing edge. The method is stopped when the tracking dye reaches the anode buffer strip (after approximately 60–65 Vh).

Sample preparation

Prepare the sample as follows:

Sample buffer: 10 mM Tris/HCl, 1 mM EDTA, pH 8.0.

Sample treatment: To the sample add SDS to 2.5% and β-mercaptoethanol to 5.0%. Heat at 100°C for 5 minutes. Add bromphenol blue to approximately 0.01%. Any insoluble material must be removed by centrifugation to prevent streaking patterns in the developed gel.

Also, you may have to adjust the salt and sample concentration. Some guidelines concerning this are described below.

Salts

The effect of salt on SDS-PAGE with PhastGel gradient media will depend on the concentration of the salt, the type of salt, and the pH of the sample. Generally, high salt concentrations (0.7 M) in combination with low pH will adversely affect results. Some salts have stronger effects than others. NaCl, for example, may be used in concentrations as high as 0.75 M for samples with a pH between 7 and 10, whereas, NaAc in concentrations as low as 0.35 M will distort results for the same pH interval. Samples containing too much salt must be diluted or desalted. For more information about the effects of salts on SDS-PAGE see (4).

Sample concentration

The sensitivity of your development technique and the volume of the sample applied to the gel will determine the lower limit of your sample concentration. Generally, the sample must contain 20 to 30 ng of each protein/μl for Coomassie staining, and at least 0.3 to 0.5 ng of each protein/μl for silver staining. Samples containing more than 2 μg/μl (Coomassie) or 50 ng/μl (silver) of each protein can overload the gel and distort results.

Methods

Tables 1 and 2 give the running conditions for one gel. Methods are always programmed for one gel. When you start the method, you enter the number of gels to run. If you enter 2, PhastSystem automatically adjusts the current and power so that both gels run under the same conditions according to the programmed method. See the Users Manual of PhastSystem for details.

The methods in tables 1 and 2 contain two steps; the first step is for electrophoresis; samples are applied to the gel and the protein components separate according to their size. The approximate electric field conditions in the gel during the run are given in figure 2.

The second step (optional) may be programmed to reduce the risk of proteins migrating off the gel should the method end when you are beyond hearing distance of the alarm. The second step is programmed for 0 Vh, so the alarm will sound immediately after step 1. The method will continue to run, with low voltage, until you stop the method. See the Users Manual for details.

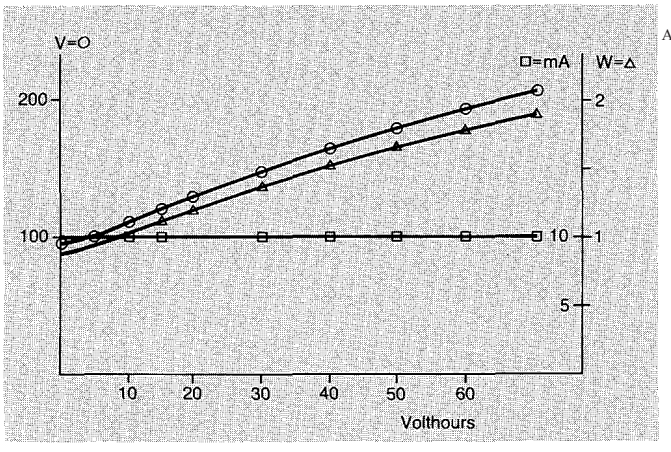


Fig. 2. Approximate electrical conditions in the gel for SDS-PAGE with PhastGel gradient media.

Table 1. Optimized method for SDS-PAGE with PhastGel gradient 10—15 to program into the separation method file of PhastSystem (given as method 1).

SAMPLE APPL. DOWN AT			1.1	1 Vh	
SAMPLE APPL. UP AT			1.1	10 Vh	
SEP 1.1	250 V	10.0 mA	3.0 W	15°C	60 Vh[1]
SEP 1.2	50 V	0.1 mA	0.5 W	15°C	0 Vh[2]

[1] This is based on runs using commercially prepared and crude extract proteins. You might have to adjust this time to suit your application. The run should be stopped when the tracking dye reaches the anode buffer strip.

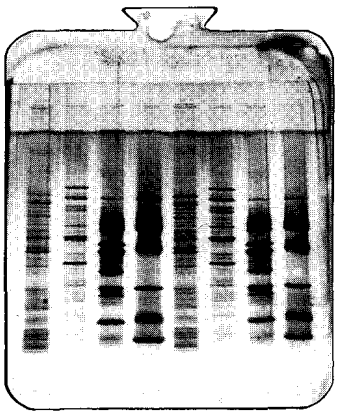
[2] This step is optional. Its purpose is to reduce the risk of proteins migrating off the gel should you miss the alarm that marks the end of the method (after step 1).

Table 2. Optimized method for SDS-PAGE with PhastGel gradient 8—25 to program into the separation method file of PhastSystem (given as method 2).

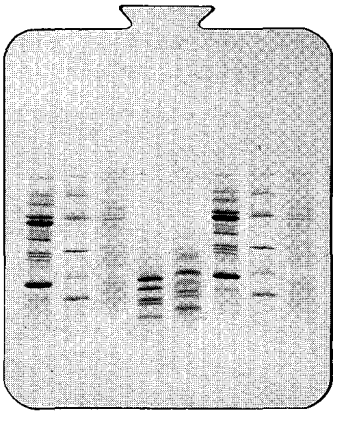
SAMPLE APPL. DOWN AT			2.1	1 Vh	
SAMPLE APPL. UP AT			2.1	10 Vh	
SEP 2.1	250 V	10.0 mA	3.0 W	15°C	65 Vh[1]
SEP 2.2	50 V	0.1 mA	0.5 W	15°C	0 Vh[2]

[1] See the note in table 1 above.

[2] See the note in table 1 above.



Example of SDS-PAGE with PhastGel gradient 10—15. The gel was silver stained using the method described in development technique file number 210. From the left the samples are: *E. coli,* Acholeplasma laidlawii, beef extract, Amersham BiosciencesLMW calibration kit proteins, and repeat starting with *E. coli.*



Example of SDS-PAGE with PhastGel gradient 8—25. The gel was Coomassie stained using the method described in development technique file number 200. The samples are, starting from the left: beef extract, Pharmacia LMW calibration kit proteins, *E. coli,* Pharmacia PMW calibration kit proteins, cleaved albumin, beef extract, LMW calibration kit proteins, and *E. coli.*

References

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