Native PAGE

Separation Technique File No. 120

PhastSystem™

This technique file describes an optimized method for native polyacrylamide gel electrophoresis (native PAGE) with PhastGel™ gradient 8–25 and PhastGel gradient 10–15 using PhastGel native buffer strips. The method has been optimized using crude protein extracts and commercially available proteins. Therefore, it is generally applicable and offers a good starting point for developing methods for specific applications.

This file gives only specific method information. Detaled 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

Native PAGE is one of the most powerful techniques for studying the composition and structure of native proteins, since both the conformation and biological activity of proteins remain intact during this technique (1). It is occasionally used for molecular weight (MW) measurements; however, SDS-PAGE is easier and in most cases more reliable than native PAGE for this application. Often, it is difficult to find standard proteins that resemble the shape, partial specific volume and degree of hydration as the native protein under investigation (2, 3).

Gradient gels for native PAGE sharpen the protein bands and allow complex mixtures of proteins to be separated on a single gel. With PhastSystem and PhastGel gradient media, native PAGE is fast, reproducible, and convenient; separations take approximately 60 minutes, are run under exact, programmed conditions, and require no buffer preparation.

PhastGel gradient media

PhastGel gradient media for native PAGE, comprises three polyacrylamide (PAA) gels, PhastGel gradient 10–15, PhastGel gradient 8–25 and PhastGel gradient 4–15, and PhastGel Native buffer strips.

Table 1. PhastGel Gradient media and Buffer Strips for SDS and Native PAGE

	Gradient 4–15	Gradient 10–15	Gradient 8–25	PhastGel SDS	Buffer Strips Native
Gel description					
Dimensions (mm)	43 × 50 × 0.45	43 × 50 × 0.45	43 × 50 × 0.45	41 × 10 × 6	41 × 10 × 6
Gel material	Acrylamide	Acrylamide	Acrylamide	3% Agarose	3% Agarose
Polyester Backing	GelBond	GelBond	GelBond		
Stacking zone					
Length (mm)	13	13	13		
Composition (% T/%C)	4.5/3	6	6		
Separating zone					
Length	32	32	32		
Composition (% T/%C)	5–15/1–2	10–15/2	8–25/2		
Buffer	A	A	A	B	C
Shelf life (months)	9	9	9	12	12
Storage temp (°C)	4–8	4–8	4–8	4–8	4–8

Buffers
A: 0.112 M Tris, 0.112 M Acetate, pH 6.4
B: 0.20 M Tris, 0.20 M Tricine, 0.55% SDS, pH 8.1
C: 0.25 M Tris, 0.88 M L-Alanine, pH 8.8

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In this technique file we will only discuss PhastGel gradient 10–15 and PhastGel gradient 8–25. For native PAGE in PhastGel gradient 4–15, please see Separation Technique File No. 130.

Molecular weight range

The gradient in the separation gel determines the molecular weight range that is possible to analyze in one run. PhastGel gradient 8–25 is designed to give a linear relationship between a protein's migration distance and the logarithm of its molecular weight for native, globular proteins between 50 000 and 750 000. Proteins above 750 000 are too large to enter the gradient gel. Proteins below 50 000 may appear on the developed gel, but their migration distance may not be in linear proportion to log MW.

Similarly, PhastGel gradient 10–15 gives a linear relationship between a protein's migration distance and log MW for the MW range 90 000 to 700 000 for native globular proteins.

The migration distance of non-globular proteins or proteins with extreme charges (for example, IgG) may not be proportional to log MW.

Principle of the method

At the start of the run, the buffer from the buffer strips migrates into the gel. The leading and trailing ions (acetate/L-alanine) form a boundary that migrates through the gel leaving behind a region of uniform voltage and constant pH (pH 8.8). As this boundary passes the point of sample application (after 10 Vh) the proteins are applied to the gel.

The pH in the gel is 8.8 so proteins with isoelectric points (pI) below approximately 8.5 take on a net negative charge and migrate through the homogeneous stacking gel zone.

When they reach the stacking/gradient gel interface, their mobility is drastically reduced due to the sudden decrease in pore size. The proteins separate in the gradient gel zone according to their size and charge.

The gradient sharpens the proteins bands since the advancing edge of the protein is retarded more than the trailing edge. After approximately 280 Vh the banding pattern remains relatively constant, but the proteins never stop migrating until the method is stopped. Figure 1 shows the migration distance of proteins plotted as a function of time.

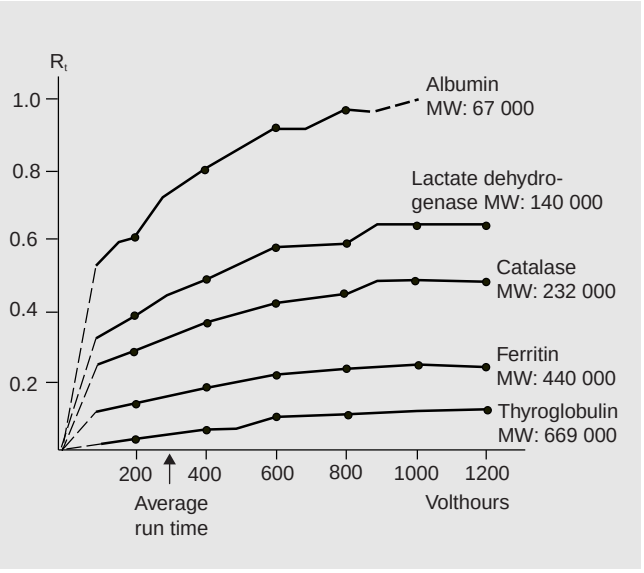

Fig. 1. Migration distance (R_f) of the HMW Calibration Kit proteins plotted as a function of time. R_f is the migration distance expressed as a percentage of the total gel length.

Sample preparation

You may need to add protease inhibitors to your samples to preserve the biological activity of the sample proteins. Any insoluble material must be removed by centrifugation to prevent streaking patterns in the developed gel. Besides these two points, the salt and sample concentration might have to be adjusted. Some guidelines concerning this are described below.

Salts

The effect of salt on native 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 (1 M) in combination with low pH (pH 4) will adversely affect results. NaCl, for example, may be used in concentrations as high as 0.75 M and NaAc as high as 1.0 M in the pH range 7 to 10. At lower pH values, results will be distorted with these salt concentrations. Samples containing too much salt must be diluted or desalted.

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 1 to 5 ng of each protein/µl for silver staining. Samples containing more than 2 µg/µl (Coomassie) or 100 ng/µl (silver) of each protein can overload the gel and distort results.

Methods

Table 2 gives the running conditions for one gel. Methods are always programmed for one gel, regardless of whether you plan to run one or two. 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 two gels will run under the same conditions according to programmed method. See the Users Manual of PhastSystem.

The method contains three steps. The moving boundary forms during the first step and starts to migrate through the gel. In the second step, the samples are applied while the current is reduced to 1.0 mA (to prevent streaking patterns).

In the third step, the current is raised again and the proteins separate according to their size and charge. The electrical conditions in the gel during the run are given in Fig. 2.

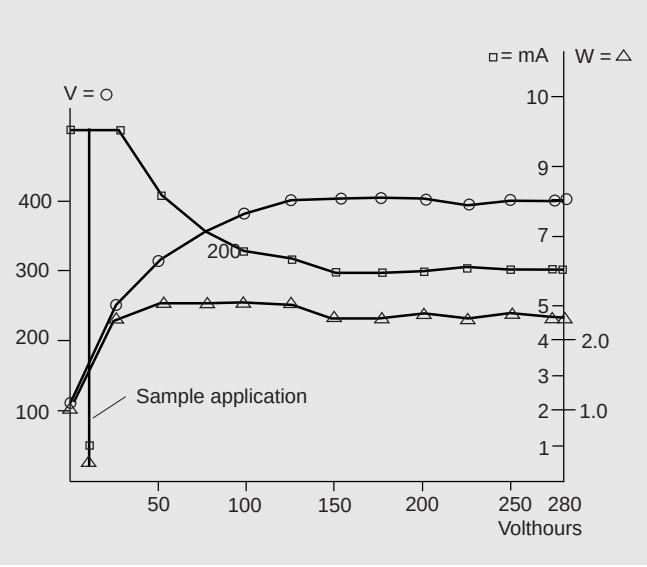

Fig. 2. Approximate electrical conditions in the gel plotted as a function of time for native PAGE in PhastGel gradient media.

Table 2. Optimized method for native PAGE in PhastGel gradient media to program into the separation method file of PhastSystem (given as method 1).

SAMPLE APPL. DOWN AT				1.2	0 Vh
SAMPLE APPL. UP AT				1.2	2 Vh
SEP 1.1	400 V	10.0 mA	2.5 W	15˚C	10 Vh
SEP 1.2	400 V	1.0 mA	2.5 W	15˚C	2 Vh
SEP 1.3	400 V	10.0 mA	2.5 W	15˚C	268Vh[1]

[1] This is based on runs using commercially prepared and crude extract proteins. You might have to adjust this time to suit your application.

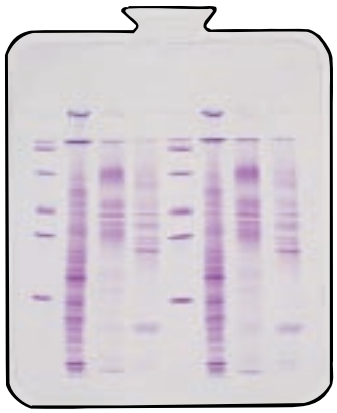

Fig. 3. Example of native PAGE with PhastGel gradients 8–25. The gel was Coomassie stained as described in development technique file number 200. Starting from the left, the four repeating samples are: HMW Calibration Kit, proteins, *E. coli*, salmon extract, and coalfish extract.

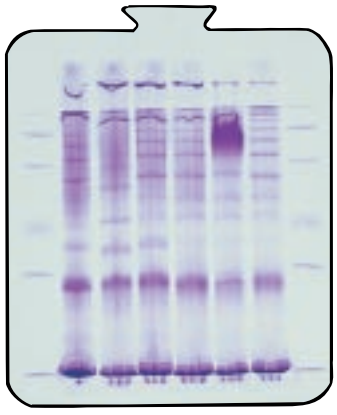

Fig. 4. Example of native PAGE with PhastGel gradients 10–15. The gel was Coomassie stained as described in development technique file number 200. The first and last lanes are HMW Calibration Kit proteins, and the samples in the middle are human serum proteins.

References

1. Disc Electrophoresis II: Methods and Application to human serum proteins: *Ann. N. Y. Acad. Sci. 121 (1964) 404–427*, Davis, B. J.

2. Molecular weight measurement and the use of gel concentration gradients. In *Electrophoresis. Theory, techniques and biochemical and clinical applications*, Peacocke, A. R. and Harrington, W. F. (editors), *Claréndon Press, Oxford, 1981, pp. 63–80*, Andrews, A. T.

3. An introduction to polyacrylamide gel electrophoresis. In *Gel electrophoresis of proteins: A practical approach*, Hames, B. D. and Rickwood, D. (editors), *IRL Press Limited, London, Washington DC, 1981, pp. 4–14.* Hames, B. D.

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