Native PhastGel Electrophoresis

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Summary

The purpose of the Native PAGE technique is to determine the homogeneity of a protein according to its banding pattern on the gel.

Materials/Reagents/Equipment	Vendor
Phast System	GE Healthcare Phone #: 1-800-526-3593
PhastGel buffer strip holder	
PhastGel gradient 8-25%	Catalog # 17-0542-01
Native buffer strips	Catalog # 17-0517-01
Distilled Water	
Forceps	

Procedure

1. Turn Phast System on (power switch located on back of the Phast System) and set the temperature according to the temperature of the first step in the method you plan to run. SEP stand by temperature = 15° C.
2. Check that the red levers are in the up position. This will allow the proper spacing for this gel.
3. Raise electrode assembly and sample applicator arm to the vertical position.
4. Wipe off separation bed with a moist, lint-free cloth to remove dust or particles.
5. Take the gel from the refrigerator and use scissors to cut the package along the 3 sides (indicated by the dotted lines).
6. Use forceps to remove the gel from the package; use the plastic tab of the gel backing as a handle. (The thin plastic film on gel surface protects gel from contaminants and from drying.)
7. Use either a waterproof pen to mark underside of the gel or cut an edge of the gel for identification.
8. Place the gel on a hard surface and bend plastic tab up using forceps. (This makes it easy to position and remove the gel from the bed.)
9. Place a drop of distilled water (60-75 µl) onto the middle of the gel area (outlined by the red lines on the separation bed).
10. Lower the gel onto one of the gel areas so that a film of water, free from air bubbles, forms between the gel support and separation bed. Remove any air bubbles by sliding the gel around.
11. Position the gel so that its edges are in perfect alignment with the red outlines.

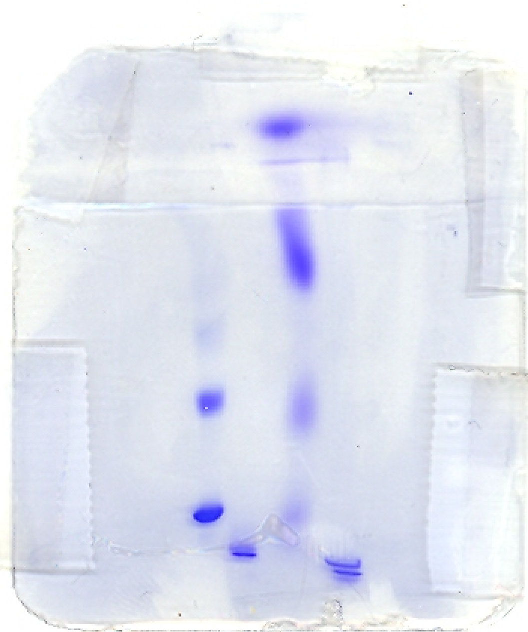
12. Remove any excess liquid with a Kimwipe.
13. Use forceps to gently lift the plastic film from the gel surface.
14. Place PhastGel buffer strip holder over the gel by first sliding the buffer strip holder forward so that the two black pins and the holes in the holder form a hinge. Lower the buffer strip holder onto the separation bed.
15. Place the native buffer strips into the compartments in the buffer strip holder; one in the anode and one in the cathode. Gloves should be worn when handling buffer strips to prevent eventual disturbance from finger proteins! (Native buffer strips can be found in the sandwich box in native buffer above the unit.)
16. Lower the electrode assembly to its initial position. Press down along the top of the electrodes to ensure that the electrodes have complete contact with the buffer strips.
17. Lower sample applicator arm.
18. Place a piece of Parafilm over the sample applicator, with the protective cover facing upwards.
19. Press down along the lane of wells to make depressions in the Parafilm. Use gloves!
20. Remove the Parafilm protective cover.
21. Place sample applicator with the Parafilm on a table so that the depressions can be filled. Fill the depressions with a volume of sample twice the applicator capillary volume. (Make sure there are no air bubbles in these samples, as these will be drawn into the applicator capillaries.)

Sample applicator	Slots / Wells	Applicator capillary volume	Suggested sample volume per slot
8/1	8	1 μL (2-4 mg/ml)	2 μL
6/4	6	4 μL	8 μL

22. Lower the applicator to the surface of the samples. Let the samples climb up into the applicator teeth. Avoid getting samples on the sides of the applicator. This applicator is **single use and is disposable**.
23. Slide the loaded sample applicator into slot nearest cathode (closest to user).
24. Close the separation compartment lid.
25. Press “SEP start/stop” and enter the number of gels for this run. Then press do.
26. Enter the number of the method you plan to run. For Native PhastGel gradient 8-25%, the method is preset to No. 3. Then press “do.”
27. When alarm sounds, the run has ended. Press “SEP start/stop” and “do” to confirm ending process. The run takes approximately 45 minutes.
28. Open the lid, remove the comb, and lift up the sample applicator. Then lift the electrode and use a Kimwipe to rinse with water. Be sure not to rub the electrodes. Remove the buffer strip holder and place the buffer strips in the sandwich box with buffer to preserve for future use. Clean up separation bed and lower all parts onto the bed. **Be sure to lay down the electrode, sample applicator and lid one at a time, or you may break the electrodes!**

29. Proceed to staining PhastGel using coomasie stain as per regular SDS/PAGE. The gel may be dried with only a layer of membrane on the top due to the backing already on the gel.

Results



Lane 1 2 3 4 5 6 7 8

Analysis

Lane	Sample	mg/ml	μL	KDa	pI	Homogeneity (Detailed)
1						
2						
3	BSA	2	1	66		Hetero- 2 bands, 1 sharp, 1 smear
4	pB3 1068 B [4] Uncut	3.2	1	18	4.8	Homo- 1 sharp band
5	PS	3.7	1	14		Hetero- 4 bands with smear through
6	CheW	3.3	1	18	4.68	Hetero- 2 sharp bands, close proximity
7	pB3 1238 B [15] cut	2.2	1	12.8	9.7	* Did not appear
8						

*pI of gel is 8.8; samples with pI < 8.8 migrate upwards, samples with pI > 8.8 migrate downwards. pI of pB3 1238 B [15] cut > pI of the gel

Buffer for Native buffer strips:

0.88 M L-alanine, 0.25 M Tris, pH 8.8

Buffer for SDS buffer strips:

0.2 M Tricine, 0.2 M Tris, pH 8.1, 0.55% SDS