SDS-PAGE with SILVER STAINING

(Wright *et al.*, 1996)

Introduction

Because of the nature of this glycoprotein (high stability and hydrophobicity), it is difficult to solubilize and to digest for electrophoretic analysis. In addition, its high lectin binding ability prevents it from being stained with simple staining procedures, such as the use of Coomassie Blue. A procedure has been developed that utilizes the PHAST system and silver staining along with a pre-incubation step that helps digest the protein. The procedure using the PHAST system is described below, if you do not have access to this equipment, get the samples ready as directed below in steps 1 and 2, run a normal SDS-PAGE gel and stain following the outlined development method.

SDS-PAGE GEL ELECTROPHORESIS with the PHAST SYSTEM

Materials

Sample with a concentration of 2.5 ug/ul according to the total protein assay*
Gel (PhastGel® Homogeneous 12.5)
SDS buffer strips (PhastGel®)
Gel box (PhastSystem®, Piscataway, NJ)
Staining box (PhastSystem-Developer)
20 mM citrate, 1 mM EDTA solution, pH 9.3 (pre-incubation buffer)
pre-incubation buffer with 1% dithiothreitol and 3% SDS (sample buffer)
50% EtOH, 10% acetic acid
50% glutaraldehyde
0.25% $AgNO_3$
0.015% formaldehyde in 200 ml 2.5% Na_2CO_3 (Developer)
5% acetic acid
10% acetic acid, 5% glycerol
de-ionized (Milli-Q) water (dH_2O)

*Glomalin sample may be raw extract or purified by precipitation and dialysis prior to electrophoretic analysis. After freeze-drying, the sample is weighed and dissolved in water. Remember that because glomalin is a glycoprotein with other groups attached, gravimetric and protein weight do not correspond – an estimate of this relationship may be determined by calculating the percentage total protein (total protein weight from the Bradford total protein assay ÷ gravimetric weight of freeze-dried glomalin). To calculate the amount of freeze-dried protein needed, take the concentration needed for electrophoretic analysis (2.5 ug/ul) divide by the percentage total protein value and multiply by 1000 to have a mg/ml value. It is advisable to weigh out a sample that is 1.5 to 2 times the amount estimated above – it is easy to dilute this even further if necessary. Run a Bradford total protein assay on the dissolved sample and determine the appropriate dilution need for a concentration of 2.5 ug/ul.

METHODS

1) Place sample in pre-incubation buffer at a concentration of 1 ul of protein (2.5 ug protein/ul dH_2O) in 4 ul of buffer and incubate overnight at 4°C.
2) Add sample buffer to incubated samples (concentration = 10:1) and boil at 100°C for 8 min.
3) Set-up gel in PhastSystem (See directions with system as well):
    A. Start gel box cooling (Sep-stand by temp 15°C).
    B. Put tubes in labeled developer solution bottles and cover loosely with parafilm.
    C. Place 70 ul of dH_2O on the bottom of gel box.
    D. Cut open container for gel and remove plastic cover.
    E. Bend tip of gel (for easy pick-up) and place gel just on the opposing side of the water drop and lay down carefully (make sure there are no air bubbles beneath the gel or water on top of the gel).
    F. Carefully position gel within red frame.
    G. Cover with plastic housing and place SDS buffer strips in slots.
    H. Put electrode on top and press in gently.
    I. Make wells for samples with parafilm and a template.
    J. Put 4 ul of sample in parafilm wells.
    K. Use comb to draw sample up and place comb in front of buffer strips.
    L. Put on cover and start program (Sep Start).
4) After program is complete, remove gel and place in racks in developer (as directed).
5) Develop gel, using development method #2 (see below for full details) with tubes going from the various solutions as outlined in the development method. The gluteraldehyde (until it turns yellow), acetic acid and acetic acid/glycerol solutions are recycled; the water rinses are disposed of down the drain; the silver nitrate and developer solutions are combined for proper waste disposal; and the ethanol and acetic acid solution are disposed of separately.
6) After bands develop, store gel in plastic wrap at room temp.

SILVER STAINING

1) Immerse in 50% EtOH, 10% acetic acid solution for 2.0 min.
2) Remove and immerse in 8.3% glutaraldehyde (20 ml 50% glutaraldehyde diluted to 120 ml in water) for 15.0 min.
3) Remove and rinse in water for two 2.0 min. intervals
4) Immerse in 0.25% $AgNO_3$ for 13.0 min.
5) Remove and rinse in water for two 0.5 min. intervals
6) Remove and immerse in developer for 0.5 min.
7) Remove and immerse in new solution of developer for 4.0 min.
8) Remove and immerse in 5% acetic acid for 2.0 min.
9) Remove and immerse in 10% acetic acid, 5% glycerol for 3.0 min.
10) Remove and observe banding