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Protocol for Protein Analysis using Protigel™ P Gel

Introduction

Protigel™ P gel was designed to effectively separate proteins over a wide molecular weight range. Reduced or non reduced proteins can be resolved by size using this room temperature stable matrix. The gel and buffer are premixed and ready to be used, reducing hands on time and inconsistencies related to gel and buffer preparation.

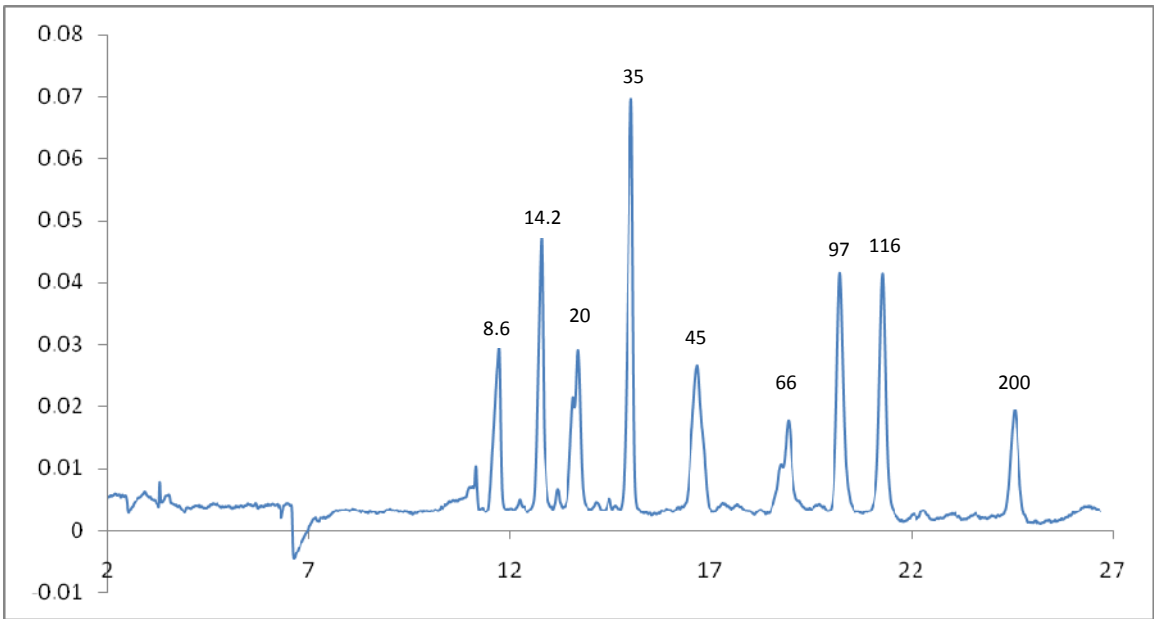

Figure 1: 9 peak protein standard separation using Protigel® P gel matrix on the Beckman® MDQ. 5 Peak Standard consists of 8.6, 20, 45, 116, and 200kDa.

Capillary Dimensions

The procedures are based on the use of a bare fused silica capillary with 75 µm i.d., 20 cm effective/30 cm total length.

Reagents needed

For best results, store unused portions of gel at 2-8°C, and warm to room temperature prior to use.

Kit Part Description
Protigel™ P protein gel
Protein capillary conditioning solution
Protein sample buffer (1X)
4N NaOH
Optional:
Protein marker (8.6, 20, 45, 116, 200 kDa)
Protein standard (8.6 kDa)

Procedure

Sample Preparation:

A. Procedure for reducing protein sample.
1. Dilute protein sample to within the specified range (see Notes) with an appropriate amount of 1X protein sample buffer for a total volume of 95 µl.
2. Add 4 µL of Protein marker.
3. Add 5 µL 2-mercaptoethanol.
4. Cap the vial and mix thoroughly then briefly centrifuge.
5. Heat the mixture at 70˚C for 10 minutes.
6. Place the vial in a room temperature water bath to cool for at least 3 minutes, centrifuge briefly.
7. Transfer 100 µL of the prepared sample into a 200 µL PCR vial and place into the sample vial holder.

B. Procedure for non-reduced protein sample.
1. Weigh out 46mg of Iodoacetamide (IAM) and add to a 1.5mL centrifuge vial.
2. Add 1mL of CE grade H_2O into the vial, cap the vial tightly and mix thoroughly.
3. Pipette volume corresponding to 100 µg of protein sample into a 200 µL PCR vial.
4. Bring the final volume of sample to 95 µL with the appropriate amount of 1X protein sample buffer.
5. Add 4 µL of Protein marker.
6. Add 5 µL of IAM alkylating reagent.
7. Cap vial tightly and mix thoroughly then briefly centrifuge.
8. Heat the mixture at 70˚C for 10 minutes.
9. Place the vial in a room temperature water bath to cool for at least 3 minutes, centrifuge briefly.
10. Place into the sample vial holder.

Instrument set up:

A. ***Important:*** **For optimal result a new 75 µm I.D., 20 cm effective/30 cm total length capillary should be installed as directed by instrument instruction manual.**

B. Set PDA detection wavelength to 220nm.

C. Reagent/Sample Vial Preparation
1. Dispense 1.5mls 1 N NaOH into a glass vial and place in inlet tray.
2. Dispense 1.5mls 0.1 N NaOH into a glass vial and place in inlet tray.
3. Dispense 1.5mls Protein capillary conditioning solution into a glass vial and place in inlet tray.
4. Dispense 1.5mls Protigel™ P protein gel into 2 glass vials. Place one vial into the inlet tray and one vial into the outlet tray.
5. Place 50µl of the reduced Protein 5 Peak standard into a PCR tube. Place the PCR tube into a sample vial holder as directed by instrument instruction manual.
6. Place 50-100 µl of samples into PCR tubes. Place into sample vial holders and load into the inlet tray as directed by instrument instruction manual.
7. Place 4 empty glass vials in the outlet tray for waste collection.

D. Capillary pre-conditioning and gel filling/pre-run*

1. Program system to rinse new capillary with 1 N NaOH at 50 psi for 15 minutes at 50°C. Collect waste in first waste vial.
2. Program system to reset capillary temperature to 25°C
3. Rinse the capillary with 0.1 N NaOH at 50 psi for 5 minutes. Collect waste in second waste vial.
4. Rinse the capillary with Protein capillary conditioning solution at 50 psi for 10 minutes. Collect waste in third waste vial.
5. Rinse capillary with Protigel™ P protein gel from inlet tray vial at 50 psi for 10 minutes. Collect waste in the fourth waste vial.
6. After capillary is filled with gel, move outlet of capillary to outlet tray vial containing Protigel™ P protein gel.

E. CE Pre-run and Separation*

1. Perform pre-run at -10 kV, reverse polarity, for 5 min with a 2.5 min ramp to stabilize current.
2. Perform sample injection at -5 kV, reverse polarity, for 20 seconds. The sample injection time may need to be adjusted, depending upon sample concentration and sample matrix.
3. Perform CE separation at -10kV, reverse polarity, for 30 minutes. Optional: 20psi may be applied to both the inlet and outlet vials if current loss is observed during separation.
4. In between each CE separation, flush capillary with Protigel™ P protein gel from inlet tray vial at 50 psi for 5 minutes. Collect waste in fourth waste vial.
5. Repeat steps E1-E4 to analyze multiple samples in a sequence.

If separation resolution/performance decreases to an unacceptable level, repeat from Step D1-D6 to recondition and refill the capillary.

F. Instrument Shutdown

1. If samples will be run within 1 day of last use, it is recommended that a shutdown method be established and run. Consult instrument owners manual for appropriate shutdown sequences.
2. If no additional samples will be run, it is recommended that a clean up method be established and run. Consult instrument owners manual for apporpriate clean up method.

**procedure tested on Beckman MDQ*

Notes

1. The Protein 5 peak standard has been reduced with 2-mercaptoethanol and should be stored at -20C. It is recommended that 50 µl aliquots be dispensed into 200 µL PCR vials prior to use. Thaw aliquot at room temperature and centrifuge briefly.
2. Protein concentration should be between 0.2-2 mg/ml.
3. Salt concentrations above 50mM might interfere with the separation. It is recommended that a desalting step be performed prior to the separation if salt exceeds this level.
4. The reagents are made with room temperature stable polymers; it is recommended, however that unused reagents at 2-8°C for optimum performance.
5. The protein marker should be added to all samples for normalization of samples run-to-run.

Need support? – contact us at tech-support-CE@aati-us.com or phone 515-296-6600