SDS-PAGE for Identifying nonmucin protein in BSM

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Abstract

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Protocol

Step 1.
Prepare the sample solutions:
1) prepare the mucin samples
2) Assign different volume for each sample, 10ul,20ul,30ul, respectively.
3) Add DDI water to the samples, let each tube has 30ul solution.
4) Add loading buffer to each sample
5) Heating them in 95 degree for 10min.

Prepare samples as indicated in the table below.

Component	Reducing	Nonreducing
Sample	5 µl	5 µl
Laemmli sample buffer	4.75 µl	5 µl
β-mercaptoethanol	0.25 µl	—
Total volume	10 µl	10 µl

Step 2.
Loading the running buffer
1) Add some running buffer into the electrophrosis cell
2) peel the green paste at the bottom and take away the top green plastic cover, set the gel in the electrophorsis cell.
3) Fill the inner and outer buffer chambers with running buffer.
4) Make sure that the level of the running buffer above the top of the gel and there is no leakage from the inner buffer chamber to the outer.

Step 3.
Performing electrophorsis
1) Load the protein sample on the gel.
2)Connect the electrophoresis cell to the power supply and perform electrophoresis
Run conditions: 150 V200 V
Run time: until the loading buffer line come to the bottom of the gel

NOTES

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Most vertical electrophoresis chambers are operated at a field strength of 10–20 V/cm for 1 mm thick polyacrylamide gels.

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Constant Voltage

Seperatign using constant voltage are often preferred because a single voltage hat is independent of the number of gels being run is specified for each gel type

Constant Power

Holding the power constant minimizes the risk of overheating

Step 4.

Staining

1) Wash gels in DI H2O

2) Remove all water from staining container and add stain (PageBlue), agitate overnight

3) Wash gel using DI water, first time 5 minutes; second time 10 minutes; third time 30 minutes; forth time, 1 hour and fifth time keep the gel in DI water agitate overnight.