PSR Protein Gel Protocol

03/28/2011

Materials

Invitrogen – Novex Mini-Cell	Can borrow PSR's
Invitrogen – 10% Bis-Tris Mini Gel	Cat #: NP0301
Invitrogen – MOPS SDS Running Buffer 20x	Cat #: NP0001
BioRad – XT 4x sample buffer	Cat #: 161-0791

Recommendations

-In lieu of the Invitrogen gels, use other pre-cast gels. Self poured gels often contain high amounts of keratin which can interfere with protein identification on the mass spectrometer.

-Keep work area free of dust, etc. This will also keep keratin levels down.

-Clean all glassware used for the procedure, the following is recommended
- -First wash: 5% Acetic Acid
- -Second wash: 20% Ethanol
- -Third wash: Deionized Water

-Please be careful not to introduce polymers into the samples. Typical sources of contamination include:
- -Low binding pipettor tips
- -Filters used on solvents, etc.
- -Using plastic pipettor tips in strong acids
- -Dirty glassware
- -Latex gloves + some organic solvents (Acetonitrile in particular)

Running Conditions

200 V for 5-8 minutes is sufficient for cleaning up a sample prior to in-gel digestion and analysis.

200V for 30 minutes is typical for a 6-fraction separation.

200V for 50 minutes on a 4-12% gradient gel gives sufficient separation for most proteins. Methods may need to be altered for proteins less than 10 kDa or greater than 100 kDa in order to get adequate separation.

Imperial Blue Protein Stain

Complete Staining

Reagents

100% diH_2O
Imperial Blue Stain (Pierce Cat # 24615)

Procedure

After electrophoresis place gel in clean tray

(4x) Add 100 mL of diH_2O for 5 min with gentle shaking

Mix Imperial Blue stain by shaking reagent bottle

Add sufficient stain to cover gel

Determine time for staining with following table (Wash should be done with diH_2O):

Sensitivity (ng)	Stain Time	Wash Time
<3	2 hours	Overnight
3-6	1 hour	1-2 hours
6-12	5-10 min	3 x 5 min

Brief Visualization

(2x) Add 100 mL of diH_2O for 5 min with gentle shaking

Add sufficient stain to cover gel, and stain for 30 minutes.

(3x) Wash with diH_2O for 10 minutes.