

PSR Protein Gel Protocol

06/15/07

Materials

BioRad - Criterion Gel Box	Can borrow PSR's
BioRad – Criterion XT Pre-cast Gel 4-12% BIS-Tris	Cat #: 345-0124
BioRad – XT MOPS 20x running buffer	Cat #: 161-0788
BioRad – XT 4x sample buffer	Cat #: 161-0791

Recommendations

-In lieu of the Criterion 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 50 min on the 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.

Gel Staining

Sypro Orange or Red stain

Uses: 1D and 2D gels of any size or format, quantitative analysis

Sensitivity: 30-40 ng

Notes: These fluorescent stains are good for quantitative work and are quite sensitive. In general, they have greater sensitivity than coomassie but are not as good as silver. General experience indicates that SYPRO orange is more sensitive than SYPRO red. However, SYPRO orange occasionally gives a strongly speckled background during imaging. Attention to cleanliness can minimize this problem. This protocol is also fast and mass spec compatibility is excellent. Highly recommended by the OHSU core lab.

Protocol

- 1) Remove the gel from the tank and place in 40% Ethanol, 2% Acetic acid, and 0.005% SDS. Minimum volume 200 mL for a 20cm x 20cm gel. Agitate gently for 20 min, repeat once. Second fix step can go overnight.
- 2) Wash gel in 2% acetic acid, 0.005% SDS. Minimum volume 200 mL. Agitate gently for 20 minutes, repeat once.
- 3) Stain with 1:5000 dilution of SYPRO orange or red in 2% acetic acid, 0.005% SDS. Minimum volume 200 mL. Agitate gently for 1 hour – sensitivity will increase up to 4 hours.
- 4) Bring the gel to the proteomics core for imaging and spot cutting.

This protocol is an adaptation from Malone et al., Electrophoresis, 2001, 22, 919-932

Gel Staining

Sypro Ruby stain

Uses: 2D gels, quantitative analysis

Sensitivity: 10-25 ng

Notes: Although the manufacturer claims levels of sensitivity near silver stain, experience suggests that this is seldom realized. However, this stain is very good in detecting proteins that generally stain poorly with silver or coomassie. Sypro Ruby fluorescent stain is good for quantitative work with 2D gels. In general, it demonstrates greater sensitivity than coomassie but is not as good as silver. Staining is achieved quickly and mass spec compatibility is excellent. This stain is recommended by the OHSU core.

Protocol

- 1) Remove gel from tank and place in 10% MeOH, 7% acetic acid. Minimum volume 200 mL for a 12cm x 12cm gel. Agitate gently for 30 min, repeat three times. Last fix step can go overnight.

- 2) Stain at least 3 hours in Sypro Ruby. Follow manufacturers recommended volumes.
- 3) Rinse the gel for 30 min in 10% MeOH 7% acetic acid.
- 4) Bring the gel to the proteomics core for imaging and spot cutting. Avoid extended delays in imaging after washing the gel.

Gel Staining

Imperial Blue Protein Stain

Reagents

100% diH₂O
Imperial Blue Stain (Pierce Cat # 24615)

Procedure

After electrophoresis place gel in clean tray

(4x) Add 100 mL of diH₂O 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 diH₂O):

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