Recommended SDS PAGE Stain Protocols

Kits like GelCode Blue from Pierce and Biosafe Coomassie from Biorad are NOT compatible for in-gel digestion and mass spectrometry analysis unless you do a fixing step first. Please see below for a modified method for GelCode Blue.

The gel must be fixed by a non-modifying, precipitation procedure such at the ethanol (or methanol)-acetic acid method used here. If the protein is not fixed in the gel as a separate step from the staining, the protein will be washed away and your results will be compromised. This is especially important for low level digests.

One may also note that handling of the gel should be minimized and that gloves should be worn at all times. These steps will minimize surface contamination of the gel.

Coomassie Stain

A. Materials and Equipment

1. **Reagents**
   a. Optima LC-MS grade water (Fisher, cat# W64 for 4 L, W61 for 1 L or W61 CS for case of 6 1L bottles)
   b. Optima LC-MS grade methanol (Fisher A456-4 for 4L)
   c. Acetic acid, sequence grade (Fisher BP1185-500)
   d. Fixing reagent 50% methanol 7% acetic acid
   e. GelCode Blue (Thermo Scientific Cat#24590)
2. **Supplies**
   a. Gel tray for staining
3. **Personal Protective Equipment (PPE)**
   a. Nitrile Gloves
   b. Lab coat

B. Procedure

a. Prepare Fixing Solution 50% methanol 7% acetic acid in water
   i. Add 500 mL of HPLC grade methanol to 300 mL of water.
   ii. Add 70 mL of acetic acid.
   iii. Adjust to 1000 mL.

3. **Coomassie Stain**
   a. Place gel in a clean tray and rinse 3 times at 5 minutes with 100 – 200 mL of Optima LC-MS grade water.
   b. Add 100 – 200 mL of fixing solution and fix the gel for 15 minutes. The gel will separate from the cassette. Pour off fixing solution.
   c. Rinse 3 times at 5 minutes with 100 – 200 mL of Optima LC-MS grade water.
   d. Mix the GelCode Blue stain reagent by gently swirling (do not shake)

e. Add 20 mL of gelCode Blue to cover the gel. Occasionally swirl the gel over the 1-hour incubation period. Cover the gel so no dust can contaminate the gel.
f. Discard stain and replace with rinse 3 times at 5 minutes with 100 – 200 mL of Optima LC-MS grade water.
g. The gel can be safely left to stain overnight for increased sensitivity.

Fluorescent Stains

Lava Purple or Deep Purple

Reagents

1. Gel-fixing Solution: mix 850ml Double-distilled water, 150ml EtOH and 10g Citric Acid powder
2. Staining Solution: mix 1L Double-distilled water 6.2g Boric Acid powder 3.85g NaOH and 5ml purple stain concentrate
3. Washing Solution: mix 850ml Double-distilled water and 150ml EtOH

Procedure:

1. Fix gels in fix/acidification solution for 2 hours.
2. Prepare staining solution with thawed stain concentrate and stain for 2 hours
3. Wash with washing solution for 45 minutes to 1 hour.
4. Acidify gels in fix/acidification solution for 1 hour.
5. Image gels on Typhoon with 610 emission filter and 532 laser*
6. Store gels in acidification solution in the dark.

Notes:

-Steps 1,3,4 can be repeated to reduce background, acidification can be extended to overnight

-1L of each solution works for 2-3 large format gels

25mls of purple stain concentrate requires:

10L Fix/Acidification: 1.5L EtOH, 100g Citric Acid

5L Staining Solution: 31g Boric Acid, 19.25g NaOH, 25ml purple stain concentrate

5L Washing Solution: 750ml EtOH

*When used in conjunction with DIGE, change to lowest wavelength laser (457nm) to avoid crosstalk. With all other stains, check other stain protocol and set up Typhoon to minimize crosstalk.

SYPRO Ruby

We recommend the SYPRO Ruby kit from Invitrogen following the package insert. The basic procedure here is optimized for standard 1 mm thick 8 cm x 8 cm mini gels. They can be easily adjusted for larger gels although all gels for mass spectrometry analysis should be 1 mm thick.

Reagents

1. Fix Solution: Prepare a solution of reagent grade 50% methanol, 7% reagent grade acetic acid. Prepare 200 mL fix solution for minigels or 1.6 L fix solution for each large format gel.
2. Wash Solution: Prepare a solution of 10% reagent grade methanol, 7% reagent grade acetic acid. Prepare 100 mL for minigels of 800 mL for each large format gel.

Procedure

1. After electrophoresis, place the gel into a clean container (clean by rinsing with ethanol or isopropyl alchohol and drying) with the appropriate volume of fix solution.
2. Agitate on a shaker for 30 minutes
3. Add 60 mL of SYPRO Ruby stain and agitate overnight.
4. Wash by transferring the gel to a clean container and add the appropriate volume of wash solution and allow gel to wash for 30 minutes.
5. Rinse the gel with ultrapure water before imaging.