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.

Standard Coomassie Stain

In this staining protocol, all reagents are prepared immediately prior to use, including the Commassie blue stain and destain solutions. It is our experience that using fresh reagents gives a darker, more consistent stain with a lower background. In addition, the use of fresh reagents will minimize any contamination (keratin) of the gel. At each stage of the procedure, the reagent is removed by vacuum aspiration. By aspirating the reagents off rather that pouring them off, gel-handling is minimized and contamination of and damage to the gel are avoided. One might note that the gel shrinks dramatically during the course of the fixing and staining and returns to its original size when equilibrated in the gel storage solution. The volumes noted are used for a single 20 cm x 20 cm gel and might be adjusted according the dimensions of the gel being stained. The procedure is carried out in ~25 cm x 40 cm glass dishes. Stainless steel pans can be used, but plastic dishes should be avoided. All containers should be rinsed with ethanol or isopropyl alcohol first to minimize keratin. Gentle agitation of the dishes at all stages of the procedure will help ensure an even treatment if the gel.

Reagents

1. *Gel-fixing solution*: Add 500mL of USP-grade 95% (v/v) ethanol to 300 mL of HPLC grade water. Add 100 mL of reagent grade acetic acid and adjust the total volume to 1000 mL with water. The final concentrations are 50% (v/v) ethanol in water with 10% (v/v) acetic acid.
2. *Gel-washing solution*: Add 500mL of HPLC-grade methanol to 300 mL of HPLC grade water. Add 100mL of reagent grade acetic acid and adjust the total volume to 1000 mL with HPLC grade water. The final concentrations are 50% (v/v) methanol in water with 10% (v/v) acetic acid.
3. *Stain*: Dissolve 0.4g of Coomassie blue R350 in 200 mL of 40% (v/v) HPLC grade methanol in water with stirring as needed. Filter the solution to remove any insoluble

material. Add 200mL of 20% (v/v) acetic acid in water. The final concentration is 0.1% (w/v) Coomassie blue R350, 20% (v/v) methanol, and 10% (v/v) acetic acid.

4. *Destain*: Add 500mL of HPLC- grade methanol to 300 mL of HPLC grade water. Add 100 mL of reagent grade acetic acid and, after mixing, adjust the total volume to 1000mL with water. The final concentrations are 50% (v/v) methanol in water with 10% (v/v) acetic acid.
5. *Storage solution*: Add 25mL of reagent grade acetic acid to 400mL of HPLC grade water. After mixing, adjust the final volume to 500mL with water. The final concentration of acetic acid is 5% (v/v).

Procedure

1. After electrophoresis, the gel is washed off the glass plates with 500 mL of the gel-fixing solution and soaked in that solution for 1hr. The purpose of this step is to gently remove the gel from the plate and begin washing the SDS-containing gel buffers out of the gel. At the end of this time, remove the solution by aspiration.
2. Cover the gel with 500mL of the gel-washing solution, and continue to fix the proteins in the gel by incubating overnight at room temperature with gentle agitation. The gel should be covered during this process to avoid contamination and to prevent the evaporation of the solution. At the end of this time, remove the solution by aspiration.
3. Cover the gel with 400mL of the Coomassie stain. Stain the gel at room temperature for 3 to 4 hr with gentle agitation. The Coomassie stain is removed by aspiration after staining.
4. Cover the gel with ~250mL of the destain solution and allow the gel to destain with gentle agitation. The destain solution should be changed several times, removing it at each change by aspiration. Continue the destaining until the protein bands are seen without background staining of the gel.
5. Equilibrate the gel in the 500mL of the storage solution for at least 1 hr. The gel should return to its original dimensions during this process.
6. Store the gel in the storage solution as needed. It might be convenient to carefully transfer the gel to a heat-sealable bag for longer-term storage.

Modified GelCode Blue Coomassie Stain

Reagents

1. Gel-Code Blue stain Reagent (PIERCE Cat. 24590 or 24592)
2. HPLC water or Mill-Q water.

Procedure

1. Fix gel in fixing solution (50:10:40 / methanol: acetic acid: H_2O) for 25 - 30 mins.
2. Wash the gel with 3 aliquots of water, shaking for 5 mins each.
3. Stain the gel in Gel-Code Blue stain Reagent for 1 hour, gently rock at room temperature.
4. Wash the gel with ddH_2O, shake about 2-3 hours, change water 3 to 4 times.
5. Store gel in 5% acetic acid solution at 4°C until in-gel digestion is performed (Gel can be stored for several weeks).

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.

Silver Stain

Silver staining is a more sensitive, but more difficult, method for visualizing protein bands in polyacrylamide gels. It should be noted that while this method of staining is more sensitive, the recovery of peptides from an in-gel digest is decreased and we DO NOT recommend silver staining for subsequent MS analysis. Silver stain is not a linear stain with regard to protein amounts. A dark spot by silver does not mean a high concentration of protein, it only means the protein stains will by silver. Similarly, a faint band by silver does not mean low levels of protein, it only suggests a protein does not stain efficiently by silver. This makes deciding if there is enough protein for MS analysis very difficult. If sensitivity is a concern then we recommend lava purple or SYPRO ruby. If you require the sensitivity of silver stain, you probably do not have enough protein for MS analysis.

In general, the key limit on the use of silver stain procedures prior to in-gel digestion and mass spectrometric analysis is the type of fixing that is used. The commonly used reagent glutaraldehyde is an excellent fixer that is used in most of the high sensitivity procedure. Glutaraldehyde, however, acts by cross-linking the protein by modifying lysine residues and make a protein intractable for proteolytic digestion. Therefore, no matter what the details of the silvering and development steps, the gel must be fixed by a non-modifying, precipitation procedure such as the ethanol (or methanol)-acetic acid method described here. In many instances, this change will reduce the overall sensitivity of the protein detection.

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

Reagents:

1. Gel-Fixer: 50% HPLC grade Methanol in HPLC grade water and, 10% acetic acid.
2. Wash: 5% HPLC grad Methanol in HPLC grade water.
3. Sensitize: 1.5 g potassium fericyanide, 3.0 g sodium thiosulfate, and 0.5 g sodium carbonate in 1 L HPLC grade water.
4. Silver stain: 1 g silver nitrate and 50 uL 37% formaldehyde (formalin) in 1 L HPLC grade water.
5. Developer: 25 sodium carbonate and 200 uL 37% formaldehyde (formalin) in 1 L water. Add the formaldehyde just prior to use.

6. Stop solution: 10 mL reagent grade acetic acid in 1 L HPLC grade water.

Procedure:

1. After electrophoresis, fix the gel by soaking in the fixing solution 30 minutes.
2. Wash with the wash solution for 15 minutes.
3. Equilibrate the gel in three changes of water, 15 minutes per change with shaking.
4. Sensitize for 2 minutes.
5. Wash with HPLC grade water for 10 minutes, four times.
6. Stain the gel for 30 min, with shaking, in the silver stain.
7. Wash the gel briefly, 1 to 2 minutes, in water.
8. Develop the gel by covering with the developer until the satisfactory visualization of the protein bands is achieved. Monitor the amount of background staining to avoid overdeveloping the gel. If the developer becomes brown and turbid, replace it with fresh developer.
9. When the desire stain is produced, stop the reaction by replacing the developer with the stop solution.
10. The gel may be stored in the stop solution.