How-To-Do-It

Method for Quick Coomassie Blue Staining of Polyacrylamide Gels

Frances T. Costa

Gel electrophoresis is a popular technique that is taught in high school and college biotechnology laboratories. It can be used to demonstrate identification of proteins by analyzing banding patterns or it can be used for DNA analysis (Hames & Rickwood 1990; Carlson 1998). One drawback about this procedure, from a teaching perspective, is that it is time consuming and cannot be completed during a three-hour laboratory session. When using conventional procedures, the time period to run the mini-gel takes a minimum of 20 minutes at 250V (constant voltage). The Coomassie Blue staining procedure will take hours or overnight to see banding patterns with high resolution (Ballog 1991; Hames & Rickwood 1990).

As a researcher, I used a nonconventional method of staining polyacrylamide mini-gels with Coomassie Blue when I analyzed the banding patterns of purified eukaryotic cell membrane proteins. This method can be applied to the community college biotechnology laboratories that use electrophoresis to separate human blood serum proteins, e.g. comparing sickle-cell anemia to normal hemoglobin (Kisiel 1980) or comparing blood serum of different animals to show evolutionary homology (Lewis 1998). A pure protein sample will show more distinct banding patterns and better resolution will be obtained when staining (Carlson 1998). The minimum protein concentration that can be analyzed using this quick Coomassie staining technique is 200 ng. This method is geared toward community colleges because some reagents used are hazardous; however when used properly, provide a quick, safe and convenient means of staining gels and observing banding patterns during a three-hour laboratory session.

Frances T. Costa is an Adjunct Biology Instructor in the Natural Sciences Division of St. Petersburg Junior College, Clearwater Campus, Clearwater, FL 33765.

I researched the availability of quick staining methods for proteins that could be purchased from major biotechnology companies. Presently Carolina Biotechnology© has a quick staining procedure for DNA gels that adds stain to the running buffer and banding patterns which appear during electrophoresis. Promega© had a similar stain (Promega Chroma-Phor) for protein gels, but this product is discontinued. The only other quick stain available for proteins is offered by Molecular Probes© and is a fluorescent stain called Cyber-Orange stain. This quick method of staining protein gels is not an effective method for teaching labs for several reasons:

1. The stain fades within 24 hours to a couple of days and the gel needs to be photographed immediately to provide a record of the banding patterns. The photographic equipment used to photograph gels is very expensive.
2. A special UV lamp is needed to see the banding patterns. The UV lamp is another expensive item that needs to be purchased.
3. The probe is very light sensitive and special care is needed to prevent exposure to light.

If a teaching lab is already using the conventional electrophoresis method and staining with Coomassie Blue, then my staining technique is quick and cost effective, and does not require purchase of extra supplies.

The students would benefit from using this quick staining method because the electrophoresis lab would be completed in a three-hour lab session, instead of two separate sessions. The students would see the method from beginning to end and there would be continuity in their understanding of this procedure. The students would leave the lab with the gratification of seeing the banding patterns and observing their protein samples on the gel. Their gels will be stable for a long period of time, provided they are kept in covered containers and submerged in distilled water. If the gel is dried it will provide a permanent record of protein banding patterns.

Materials & Methods

Students should be asked to take the following precautionary measures:

- Students should wear gloves at all times.
- A microwave and a working fume hood are needed for this procedure. This method of staining polyacrylamide mini-gels should not be attempted if a working fume hood is not available in the laboratory in which the procedure is being performed.
- Students should be informed that high voltage is passing through the chamber during electrophoresis and students could be electrocuted if they do not follow proper safety precautions. The voltage should be turned off and unplugged before students open the chamber to remove the gels.

I. Preparation of Coomassie Blue Stain, Super Destain Solutions, 5X Running Buffer and 5X Sample Buffer

The Coomassie Blue Stain, Super Destain, 5X Running Buffer and 5X Sample Buffer should be prepared before this laboratory experiment is performed.

Coomassie Blue Stain Preparation

Coomassie Blue Stain is prepared using the following recipe: Dissolve 0.25g of Coomassie Brilliant Blue (Brilliant Blue R.250) into 90 ml of Super Destain Solution.

Super Destain Stock

Under a fume hood, mix 400 ml of distilled deionized water, 500 ml of methanol and 100 ml of glacial acetic acid.

5X Running Buffer Preparation (pH 8.3)

Dissolve the following into 600 ml of deionized water: 9 g Tris Base, 43.2 g glycine, and 3 g SDS. Running Buffer can be stored at room temperature and gel should run with room temperature buffer.

5X Sample Buffer Preparation

Add the following ingredients to 3.8 ml of deionized water: 1.0 ml of 0.5 M Tris-HCl (pH 6.8); 0.8 ml of glycerol; 1.6 ml of 10% (w/v) SDS; 0.2 ml of .5M DTT and .4 ml of 1% (w/v) bromophenol blue.

II. Preparation of Polyacrylamide Gels

BioRad© Mini-Protean II Electrophoresis Kit was used with spacers that had a thickness of 1.5 mm. A discontinuous polyacrylamide gel was casted. This gel consists of an upper stacking gel and a lower separating gel. The stacking gels act to concentrate large sample volumes, resulting in better band resolution than is possible when using a gel without an upper stacking layer (Laemmli 1970). It is safer and easier to use a 30% stock Acrylamide/Bis solution that can be purchased from BioRad©. Acrylamide in solution is safer than in powdered form; however, when using this reagent, take special care such as wearing gloves.

Preparation of 12% Separating Gel

Add the following to 3.35 ml of deionized water: 2.5 ml of 1.5 M Tris-HCl (pH 8.8); .1 ml of 10% (w/v) SDS stock; 4 ml of Acrylamide/Bis (30% stock); .050 ml of 10% ammonium persulfate and 5 µl of TEMED. While the separating gel is in liquid form, fill the gel chamber and leave approximately 1.5 to 2 cm space at the top for the stacking gel (BioRad 1991).

Preparation of 4% Stacking Gel

Add the following to 6.1 ml of deionized water: 2.5 ml of 0.5 M Tris-HCl (pH 6.8); .1 ml of 10% (w/v) SDS; 1.33 ml of Acrylamide/Bis (30% stock); .050 ml of 10% ammonium persulfate and 10 µl of TEMED (BioRad 1991). While the stacking gel is in liquid form, fill the gel chamber to the top and insert the combs. The gel will solidify in approximately 30 minutes and the combs will mold loading wells into the gel.

Procedure

Step 1. Prepare the following solutions as described in the **Materials & Methods** section, before the three-hour laboratory session begins:

- Coomassie Blue Stain
- Super Destain
- 5X Running Buffer
- 5X Sample Buffer

Step 2. A discontinuous polyacrylamide gel will be prepared by students as described in the **Materials & Methods** section. While the students are waiting for the gels to solidify (30 minutes), they can prepare their protein samples.

Step 3. Preparation of Protein & Molecular Weight Marker Samples

The protein samples are added to the 5X Sample Buffer using a 1:4 ratio of sample buffer to protein sample. For example, add 20 µl of a protein sample with a concentration of 200 ng, to 5 µl of 5X Sample Buffer. The sample is prepared in a microcentrifuge or Eppendorf tube that contains lid locks (prevents the lids from popping when boiling the sample). The protein sample is then heated for 3 minutes in a dry bath (using a heat block) or boiled 1 to 3 minutes at 95° C.

Depending upon the molecular weight of the protein samples, high or low molecular markers are prepared and used as standards (BioRad© low molecular markers have a molecular range of 10 to 100 Kdaltons and the high molecular markers have a range of 40 to 250 Kdaltons). Add 1 µl of molecular weight marker (they are very concentrated) to 100 µl of 5X Sample Buffer. Heat these samples for 1 to 3 minutes before loading onto the gel.

Step 4. Loading the Samples onto the Gel

Carefully remove the combs from the gels and place the gels into the electrophoresis chamber. Fill the chamber with 5X Running Buffer, submerging the gels in the buffer. Load 10 µl of the prepared molecular weight marker into Well #1. Load 15 µl of protein samples into the remaining wells (use one well per sample and take care not to contaminate adjacent wells with different samples).

Step 5. Electrophoresis Run

The gels are now loaded and ready to run. Tightly place the lid onto the chamber. Turn the voltage on (250V, constant) and check to see that the buffer and voltage are flowing through the chamber. A good indication that the buffer and voltage are flowing through the chamber is the formation of bubbles from the buffer at the bottom of the chamber.

CAUTION: Electric voltage is in use. To avoid electrocution, do not open the chamber without first turning off the voltage supply and unplugging the apparatus.

Step 6. Quick Coomassie Blue Staining Procedure

After completion of the electrophoresis run, gels are carefully placed in a medium-sized microwave container (at least two inches in height) and filled with Coomassie Blue Stain. The stain should cover the entire gel. The container should have a lid that fits properly, completely sealing the container. The lid will not pop open while microwaving the gel if the lid fits properly on the container. The container is then placed in the microwave and the microwave is set at the highest power. The gel is microwaved for 1 minute or until the Coomassie Blue Stain starts to boil. The covered microwave container is removed and brought to the fume hood that is in proper working order. The lid of the container is opened under the fume hood **ONLY**. The gel sits in the Coomassie Blue Stain for approximately 5 minutes. The Coomassie Stain is poured into a container and can be used again to stain future gels. With the gel still in the same microwave container, gently rinse the gel with tap water, taking care not to break the gel. (The sink should have a drain stopper to ensure that the gel does not go down the drain.)

Step 7. Quick Super Destain Procedure

The gel is destained using the Super Destain solution. Pour Super Destain over the gel, covering the whole gel. Place the lid to the microwave container on tightly. (**CAUTION:** Fumes from the microwaved Destain solution are hazardous if inhaled.) Place the container into the microwave and microwave the gel again at high power for 1 minute or until the Destain starts to boil. Remove the container from the microwave and bring it to the fume hood. Remove the lid under the fume hood **ONLY**. The gel will start to destain and should sit in the Destain solution for 15 minutes. The Destain solution can be changed after 15 minutes by pouring off the old Destain and adding new Destain solution. The Destain solution can only be used once and should not be recycled. Microwaving the gel again at this point is not necessary, and protein banding pattern should be visible in approximately 30 to 40 minutes.

Summary

When conducting biotechnology labs, time is limited and completion of the whole electrophoresis procedure, including staining, is impossible to accomplish during one laboratory period. I described a nonconventional Coomassie Blue staining procedure that allows the students to prepare, run and stain a polyacrylamide mini-gel of protein samples in a three-hour lab session. The total time for this staining procedure is as follows:

A. If the Coomassie Blue Stain, Super Destain, 5X Running Buffer and 5X Sample Buffer are prepared in advance, it takes one hour to prepare and solidify the mini-gel (while the gel is solidifying, the students will prepare the protein sample).
B. It takes 30 minutes to run the mini-gel at 250V (constant voltage).
C. Coomassie Blue staining and microwaving takes 5 minutes.
D. Complete destaining takes 30 to 40 minutes.

Total procedure time = 2 hours and 10 minutes. There is even time left over, 30 to 40 minutes, for students to learn a new procedure. This staining technique can easily be incorporated into teaching labs and the quick results will enhance the understanding of gel electrophoresis of proteins.

References

BioRad. (1991). *Mini-Protean II Electrophoresis Cell Instruction Manual.* Hercules, CA: BioRad Laboratories.

Bollag, D. & Edelstein, S. (1991). *Protein Methods.* New York: Wiley-Liss, Inc.

Carlson, S. (1998). Sorting molecules with electricity. *Scientific American, 279*(6), 110–111.

Hames, B.D. & Rickwood, D. (1990). *Gel Electrophoresis of Nucleic Acids. A Practical Approach,* 2nd ed. Oxford: IRL Press.

Hames, B.D. & Rickwood, D. (1990). *Gel Electrophoresis of Proteins. A Practical Approach,* 2nd ed. Oxford: IRL Press.

Kisiel, D. (1980). *Introductory Laboratory Exercises for Human Anatomy & Physiology.* Wayne, NJ: Avery Publishing Group, Inc.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature, 227,* 680.

Lewis, C. (1998). *Laboratory Inquiry Programs for Modern Biology II,* 3rd ed. Garden City, NY: Avery Publishing Group, Inc.