

Chapter 17

Fluorography of Polyacrylamide Gels Containing Tritium

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Introduction

Fluorography is the term used for the process of determining radioactivity in gels and other media by a combination of fluorescence and photography. Since most of the radiation of a low energy emitter will largely be absorbed by the gel, in the technique of fluorography a fluor (e.g., PPO) is infiltrated into the gel where it can absorb the radiation and re-emit light that will pass through the gel to the film. The resulting photographic image is analogous to an autoradiograph, but for a low energy β -emitting isotope like ^3H , the sensitivity of fluorography

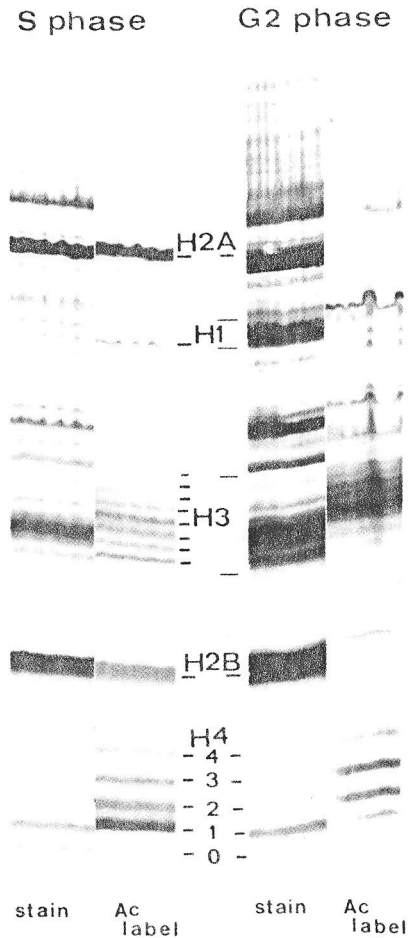


Fig. 1. This shows two examples of fluorography of protein bands labeled with ^3H . Basic nuclear proteins were isolated from the slime mold, *Physarum polycephalum*, pulse-labeled with ^3H -acetate in either S phase or G_2 phase of the naturally synchronous cell cycle. The proteins were analyzed by acrylamide gel electrophoresis in acetic acid, urea, and Triton X-100. After electrophoresis, the gel was stained with Coomassie blue, photographed, and then fluorographed. Individual lanes of the gel image were cut from the photograph (negative) and the fluorograph and then printed side-by-side to give the figure shown.

Notice that the stain patterns of the two lanes are practically identical, except for the loading, while the radioactivity patterns show major differences, for example, the absence of label in histones H2A and H2B in G_2 phase (4).

is many times the sensitivity of autoradiography. The fluorograph may be used directly, as a qualitative picture of the radioactivity on the gel. It may also be used to locate radioactive bands or spots that can then be cut from the original gel for further analysis, or be scanned to give quantitative information about the distribution of radioactivity. Figure 1 shows an example of a gel that was stained with Coomassie blue and then fluorographed. Notice that there is no loss of resolution in the fluorography of thin gels of normal size.

The procedures described here are based on those described by Laskey and Mills (1), Bonner and Laskey (2), and Randerath (3).

Materials

1. -70°C freezer
2. Film cassette, preferably with enhancing screen
3. Small photographic flash unit
4. Gel dryer
5. Film developer
6. Fixing solution: 7% acetic acid, 20% methanol in distilled water.
7. Acetic acid, 25% (v/v)
8. Acetic acid, 50% (v/v)
9. Acetic acid (glacial), 100%
10. PPO solution: 20% (w/v) PPO (2,5-diphenyloxazole) in glacial acetic acid.
11. Film: Kodak XAR-5

Method

1. After electrophoresis, remove the gel from the apparatus and fix by soaking in Fixing solution for 1 h. If required, the gel may then be stained with Coomassie blue, destained, and photographed.
2. Dehydrate the gel by shaking it for 10 min each in 25% acetic acid, 50% acetic acid, and glacial acetic acid.

3. Completely cover the gel with not less than 4 gel volumes of PPO solution and shake the gel in the PPO solution for 2 h.
4. Transfer the gel gently and evenly to a dish of distilled water and shake for 2 h. In water, PPO precipitates so that the gel turns opaque white.
5. Dry the gel completely (*see* Chapter 16), using Saran wrap on one side, on Whatman 3MM paper.
6. Open a film cassette and place the dry gel (after removal of the Saran wrap) in the cassette with the enhancing screen if used. Leave the top of the cassette beside the part with the gel in it with the white inner lining facing up.
7. In complete darkness, place a sheet of film on the white lining of the open cassette and pre-flash it. (*see* Note 5)
8. Assemble the cassette with the film directly on the gel. Wrap the cassette with aluminum foil and place in the -70°C freezer.
9. Remove the cassette from the freezer after the appropriate exposure time. (*see* Note 7) and allow to warm up for about 2 h at room temperature.
10. In complete darkness, open the cassette and develop the film.

Notes

1. Other fixing solutions may be used in step 1. For example, formalin can be used to fix peptides covalently in the gel.
2. Coomassie blue staining gives minimal color quenching, but very heavily stained bands may show reduced efficiency for fluorography. Amido black gives more color quenching and is not recommended. Silver staining has not been tested. It is possible to re-swell the gel after fluorography and stain it then, but some loss of resolution occurs.
3. The times given in steps 2 and 3 are for 0.5–1.5 mm thick gels containing 15% acrylamide. Thicker or more concentrated gels will require longer periods in all solutions.

4. Since PPO is expensive, it is normally recycled as follows: Set up four 4 L Erlenmeyer flasks with a large stirring bar and about 2.5 L of distilled water in each. Add about 0.25 L of used PPO solution, slowly, to each Erlenmeyer, stirring continuously. The PPO crystallizes out. Collect the PPO by filtering the solutions. Dry the crystals at 20°C for 2–3 d. Dissolve the crystals in a minimum volume of ethanol and precipitate, filter, and dry again. Finally, dry the PPO in a vacuum oven for about 1 wk, breaking up lumps at intervals. Glassware that was used for PPO should be rinsed in ethanol before washing.
5. *Pre-flashing* is used to improve the sensitivity and linearity of response of the film (1). Use a small, battery-operated, photographic flash unit. Tape a red filter and a diffusing screen of Whatman 3 MM paper over the flash window. Experiment with the number of layers of paper required to give an absorbance of about 0.1 when the film is pre-flashed. To pre-flash, hold the flash unit directly above the film, about 60 cm away, and press the manual flash button. Use a procedure that you can easily reproduce in the dark. Note that the first flash after the flash unit is switched on may be different from subsequent flashes, so avoid using the first flash. The white inner lining of the film cassette provides a uniform, reproducible background for pre-flashing.
6. Be careful not to place the cassette near penetrating radiation from sources such as a ^{32}P or ^{125}I autoradiograph or radioactive samples. This radiation will fog the fluorograph. Make sure the cassette is light-tight, too.
7. Recommended exposure times are of the order of 24 h for 1000–10,000 dpm ^3H and correspondingly longer for lower amounts of radioactivity. Exposure times of several months do not give a significant increase in background. Sensitivity for ^{14}C is reported to be about $10\times$ that for ^3H . Note that high acrylamide concentration such as the 50% gels used for peptide analysis severely quench the fluorescence and drastically reduce the efficiency of fluorography.
8. An automatic developer is most convenient. Manual development is described in Vol. 2, Chapter 8.

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