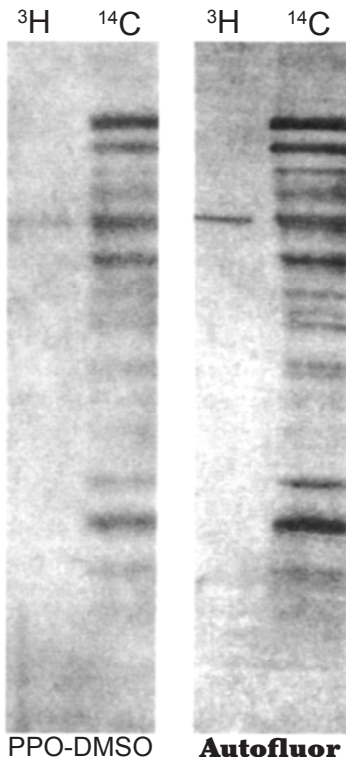


AutofluorTM

Autoradiographic Image Intensifier



Documented...
Autofluor is
Superior!

-- Perng (1988), *Analytical
Biochemistry*, **173**, 387-392

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Autofluor[™]

DIRECTIONS FOR USE

A. GELS

1. After staining, fix the gel with 5% glacial acetic acid, 5% isopropyl alcohol, and 90% water. Fix for 15 to 20 minutes. Pour off fixing solution and discard according to radioactive disposal procedures.
2. Rinse the gel in a continuous flow of tap water for 15 minutes to assure the complete removal of acetic acid residue.

[To prevent crystal formation, it is important that the gel be thoroughly rinsed after fixing. Should the gel develop white crystals on contact with **Autofluor**, dissolve the precipitate by soaking the gel in a solution of 1g sodium carbonate/100ml water or 1X TRIS Buffer. Soak the gel in **Autofluor** until the white precipitate dissolves. Repeat from the beginning of step two.]

3. Cover gel with **Autofluor** until the depth of **Autofluor** is twice the thickness of the gel. Gently agitate in **Autofluor** for 30 min/mm of gel thickness. Pour off remaining **Autofluor** and retain for future use. Label reserved material as radioactive. **Autofluor** may be reused several times before a diminishing response is observed.
4. DO NOT WASH GEL. Place directly on filter paper and dry on gel dryer under heat (80°C) and vacuum.
5. The gel will have a white to light tan sparkling appearance similar to freshly fallen snow.
6. Place on film and expose at -76°C. Due to the higher light output of the **Autofluor** phosphor, less exposure time is needed for gels treated with **Autofluor** than for gels treated with PPO/DMSO. Sufficient exposure time for a 5000 dpm/band is 24 hours. Overexposure of the film will cause the bands to become fuzzy and resolution to be lost.
7. Develop film according to manufacturer's instructions.

B. PAPER CHROMATOGRAPHY AND TLC PLATES:

1. Spray twice or dip plates in **Autofluor** and allow to dry.
2. Place on film and expose at -76°C .

TO MAXIMIZE AUTOFLUOR EFFICIENCY:

- If gels crack or stick during drying, add 0.5% (5ml/liter) of glycerol directly to the Autofluor before using.
- Since **Autofluor** is inducted into the gel by crystallization *in situ* as opposed to precipitated, it is advantageous to form the smallest crystals possible. This is accomplished by drying as quickly as possible under the strongest vacuum possible. A vacuum pump with a good seal on the dryer is preferred over a “house vacuum.” After the gel appears dry, turn off heat and continue vacuum for another 1/2 hour.

APPROXIMATE FILM EXPOSURE TIME:

ISOTOPE	dpm/band	Beq./band	EXPOSURE(hr)
³ H	500	8.3	48-72
³ H	5000	83	24
¹⁴ C/ ³⁵ S	300	5.0	24
¹⁴ C/ ³⁵ S	1000	17	8-12
³² P	500	8.3	12

NOTE: 300dpm=5dps(Beq.)=0.14nCi

STORAGE:

- Store at room temperature, out of direct sunlight. Keep from freezing. At temperatures less than 20°C precipitation of the water soluble phosphor may occur. Warming to approximately 30°C will redissolve these phosphors.

PACKAGING: One liter amber glass bottle.

SHIPPING WEIGHT: 4 lbs./liter

- **Autofluor** is not considered a hazardous waste as per EPA regulation CFR 40 Part 261 Appendix 7 Sub-Section D.

PROBLEM-SOLVING GUIDE

This guide is organized to address the three main categories of problems that can occur in autoradiography. To use this guide, carefully examine the final film to determine the general category of the problem you are experiencing. Once the problem has been diagnosed, scan the possible sources for the most likely cause and solution.

The major types of autoradiography artifacts are:

- I. Poor Image Quality (see Table 1)
 - A. Faint Image
 - B. Poor Resolution
 - C. Patchy Image

- II. Blackening or Cloudiness of Image (see Table 2)
 - A. Fogging All Over
 - B. Fogging that Follows Gel Outline
 - C. Fogging not on Gel

- III. Sharply-Defined Images (see Table 3)
 - A. Ragged/Lightening-Like Images
 - B. Black Spots, Splash Marks
 - C. Localized, Small Black Spots
 - D. Geometrical Shading
 - E. Crescent-Shaped Marks

TABLE 1: POOR IMAGE QUALITY

PROBLEM: FAINT IMAGE

SOURCE	SOLUTION
Incorrect Exposure Temperature	Expose film at -76°C.
Incorrect Film	Consult film directions.
Exposure Time too Short for Levels of Activity Used	Increase exposure time.
Overused Developer	Use fresh processing chemicals.
No Pre-flash	Pre-flash film for autoradiography.
Isotope Activity Levels too Low	Check calculations.
Quenching of Light Due to Presence of Stain in Gel	Elute dye with ethanol.

TABLE 1: POOR IMAGE QUALITY (CONT.)

PROBLEM: POOR RESOLUTION

SOURCE	SOLUTION
Urea in Gel	To remove urea, soak gel in 6% acetic acid. Rinse thoroughly.
Poor Initial Separation	Repeat separation stage.
Poor Contact between Gel and Film	Make sure cassette is properly assembled.
Ice Crystals Develop in Wet Gels.	Dry the gel thoroughly before exposure.
Diffusion of Bands	Reduce exposure time to film.

TABLE 1: POOR IMAGE QUALITY (CONT.)

PROBLEM: POOR RESOLUTION (CONT.)

<u>SOURCE</u>	<u>SOLUTION</u>
Loss of Resolution Due to the Use of an Intensifying Screen.	Eliminate the screen and expose longer if necessary.

PROBLEM: PATCHY IMAGE

Poor Contact Between Film and Object	Using a good quality cassette will provide even pressure.
Dust on Intensifying Screen	Keep screens clean.
Uneven Gel Drying	Check for clogging in dryer vents.

TABLE 2: BLACKENING OR CLOUDINESS ON IMAGE

PROBLEM: FOGGING ALL OVER

<u>SOURCE</u>	<u>SOLUTION</u>
Pre-flash too Bright	Determine proper degree of flash required. Use Kodak Wratten filters No. 21 and 22 and vary the flash distance from the film.
Processing Chemicals too Old	Use new processing chemicals.
Light Getting into the Darkroom	Be sure to completely seal off the dark room from light.
Use of Old Film	Be sure that the film has not expired.
High Radiation Close to Film Stocks	Move the film stocks away from radiation.
Wrong Safelight/ Safelight too Close to Film	Check the wattage and filters. Move the film if it is too close to light.

TABLE 2: BLACKENING OR CLOUDINESS ON IMAGE

PROBLEM: FOGGING THAT FOLLOWS GEL OUTLINE

<u>SOURCE</u>	<u>SOLUTION</u>
Light Emission from Fluor in Substrate	Make sure to dark adapt the gel for about 35 minutes before exposing it to film.
Radioactive Material Contaminating Gel Components, or Fluorographic Reagent	Count all samples and do not use any that are contaminated.

PROBLEM: FOGGING NOT ON GEL

Film Contaminated with Processing Chemicals	Keep the dark room clean. Watch for spills.
Radioactive/ Chemical Contamination	Always clean the cassette before use.
Pressure Marks from Rollers on Processing Equipment	Film should be at room temperature before use.

TABLE 2: BLACKENING OR CLOUDINESS ON IMAGE (CONT.)

PROBLEM: FOGGING THAT FOLLOWS GEL OUTLINE

<u>SOURCE</u>	<u>SOLUTION</u>
Chemography or Chemical Fogging	This is caused by insufficient drying. Dry gels thoroughly before exposure. Also quick rinse the gels before drying. Run all control gels without activity.

TABLE 3: SHARPLY DEFINED IMAGES

PROBLEM: RAGGED/LIGHTNING-LIKE IMAGES

<u>SOURCE</u>	<u>SOLUTION</u>
Electric Charge Build-Up from Use of Plastic Wrap on Film or Gel	Discharge the static before handling. Avoid using adhesive tape on film.

PROBLEM: BLACK SPOTS OR SPLASH MARKS

Dripping Fixer on Underdeveloped Film	Clean up spills immediately.
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PROBLEM: LOCALIZED, SMALL BLACK SPOTS

Storage of Film Near Radiation	Move the film away from all sources of x-rays and gamma rays.
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TABLE 3: SHARPLY DEFINED IMAGES (CONT.)

PROBLEM: GEOMETRICAL SHADING

<u>SOURCE</u>	<u>SOLUTION</u>
Exposure of Film to Light	Seal off the dark room. Check the wattage of the bulb in the safelight.
Film Developing Unevenly	Keep the films separated and agitate films during development.

PROBLEM: CRESCENT SHAPED MARKS ALL OVER

Bending of Film before or after Exposure	Bending the film before exposure causes white crescents. Bending the film after exposure causes black crescents.
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