cytiva

Amersham Amplify Fluorographic Reagent

Product Booklet

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1 Introduction

Product code

NAMP100

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or in vitro procedures for diagnostic purposes.

Safety

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice. NAMP100 is supplied as an aqueous solution in the pH range 3.5–4.5. It may cause sensitization by skin contact. It is sufficently dilute for the effects of any harmful component to be mitigated. Prolonged skin contact or eye contact is to be avoided.

Storage

- Store at room temperature protected from direct sunlight.

- Do not freeze as this will cause the fluor to precipitate. Should this occur, the fluor may be redissolved by equilibration at room temperature, followed by shaking or stirring as necessary.

Expiry

Amplify may be stored at room temperature, protected from light, for at least six months without appreciable loss of performance.

Gels impregnated with Amplify may be stored for at least three months before appreciable loss of performance is apparent.

2 Description: Amplify Fluorographic Reagent

Fast

Following a 30-minute fixing step, 15–30 minutes soaking in Amplify is all that is necessary. The gel may then be dried and fluorographed.

Convenient

Impregnation of the gel with fluor is achieved in a one-step process. The product is aqueous-based and no laborious pre-treatment or rinsing steps are required.

Safe

Amplify is odourless and does not contain DMSO or other organic solvents.

Amplify may be used to drastically reduce exposure times for gels containing ^{3}H-, ^{14}C-, or ^{35}S-labelled samples.

Amplify does not contain any substances designated as hazardous by the EPA. It may, therefore, be disposed of down the drain provided the radioactive concentration does not exceed regulatory limits. See also MSDS.

3 Directions for use

Step	Action
1	For the detection of radiolabelled proteins separated by polyacrylamide gel electrophoresis, first fix the proteins by staining/destaining or using a fixing solution (for example isopropanol:water:acetic acid (25:65:10)[1]) for approximately 30 minutes.
2	After pouring off the fixing solution into a radioactive waste container, soak the gel in Amplify (sufficient for the gel to be freefloating) with agitation for 15–30 minutes.
3	Remove the gel from the solution and dry under vacuum at 60–80°C.
4	Hold the gel in close contact with an appropriate X-ray film ('pre-flashed'[2] if quantitation is required) at -70°C to -80°C. After exposure, the film should be developed according to the manufacturer's instructions.

1 The use of trichloracetic acid for fixing proteins will adversely affectthe performance of the fluorographic reagent.

2 For further information relating to the practical and theoretical aspects of fluorography, please see our booklet "Radioisotope detection by fluorography and intensifying screens" by Professor R. A. Laskey. Copies of this booklet are available on request from Cytiva

4 Guide to exposure times with Amplify

Exposure time varies with the method of fluorography used, amount of radioactivity in each band, intensity of band(s) required, etc.

The table below refers to dried polyacrylamide gels. The amounts of activity quoted are for 5 mm slots, assuming that all the radioactivity is present as a single component, and that exposures are made at -70°C using pre-flashed film.

Isotope	Approximate time (hours) for image to be visible[1]					
^{3}H	3.7 Bq	0.1 nCi	37Bq	1.0 nCi	370 Bq	10 nCi
	>1000		100		10	
$^{14}C/^{35}S$	0.37 Bq	0.01 nCi	1.85 Bq	0.05 nCi	18.5 Bq	0.5 nCi
	>500		100		10	
^{3}H	1.85 kBq	50 nCi	3.7 kBq	100 nCi	9.25 kBq	250 nCi
	2		1		<0.5	
$^{14}C/^{35}S$	185 Bq	5 nCi	925 Bq	25 nCi	1.85 kBq	50 nCi
	1		<0.5		<0.5	

[1] with pre-flashed film the minimum OD visible is 0.2–0.3 OD units.

Note: *1 nCi – 37 dps (Bq) = 2220 dpm*

5 Troubleshooting guide

These tables have been prepared to help in diagnosing the more commonly encountered problems in autoradiography and fluorography. Although it is not possible to diagnose every problem that may arise, careful examination of the final film image will give clues as to the origin and causes of such problems.

Generally autoradiographic and fluorographic artifacts fall into three major recognisable categories:

- Sharply-defined images on the film: see *Table A. Sharply defined images, on page 8*
- Image quality is poorer than expected: see *Table B. Image quality poorer than expected, on page 10*
- General cloudiness/blackening obscuring the developed image (fogging): see *Table C. General cloudiness/ blackening obscuring the developed image (fogging), on page 13*

Table A. Sharply defined images

Problem: Tree-like, very dark images ... spark-like or sometimes softly diffuse.

Cause	Remedy
Static	
• Successive sparking discharges of static electricity caused by peeling undeveloped film from a charged surface (plastic). • Use of plastic wrap over gel. • Use of adhesive tape on film. Removal of such tape causes a static discharge that can be visible in a darkroom.	• Static discharges tend to occur in conditions of low humidity – control humidity of darkroom. • Discharge static to earth before handling film.

Problem: Intense film blackening at points of contact with gel etc. Blackening usually confined to the gel region only.

Cause	Remedy
Chemical fogging (chemography)	
• Caused by inefficient drying of gel. Residues of chemicals in the gel especially those from fixing solutions – acetic acid and TCA. Reducing agents in biological materials in direct contact with the film can produce latent image in the silver halide crystals that develop into a plausible autoradiograph as the image normally follows contours in the specimen/gel.	• Dry gels thoroughly before exposure. Give gels a final quick rinse in distilled water before drying down. • Run control gels without activity.

Problem: Small crescent shaped marks or striations generally distributed over the film.

Cause	Remedy
Pressure marks	
• Distortion of the film base causes pressure and stress on the emulsion layer. Surface pressure and scratching can also cause a latent image to be generated in the emulsions. Black crescents are a result of bending film after exposure, white crescents are due to bending film before exposure.	• Handle film carefully. Do not bend or force the film into small cassettes.

Problem: Discrete local blackening.

Cause	Remedy
Storage of film near radiation	
• Storage of films near X-ray source. • Storage near β-emitting isotopes in a lead container.	• Keep films away from all sources of x-rays etc.

Problem: Straight lines, shading geometrical patterns. Light and dark areas.

Cause	Remedy
Exposure of film to light	
• Of sufficient intensity to fog the film usually with the intervention of a shading object like the edge of another sheet of film or the film box itself.	• Check wattage of bulb in the safelight and light-tightness of darkroom.
Uneven development	
• Films hung too close together in developer.	• Agitate films during development. Keep them well separated.

Problem: Black spots and/or splash-like marks.

Cause	Remedy
Drips of fixer on undeveloped film	
• Bad darkroom housekeeping.	• Take care to mop any processing chemical spillages immediately.

Table B. Image quality poorer than expected

Problem: Image faint

Cause	Remedy
• Wrong film used	• Consult user guide.
• Wrong exposure temperature	• Use -70°C for fluorography with Amplify.
• Exhausted developer	• Use fresh processing chemicals.
• Pre-flash omitted	• Pre-flash film for fluorography.
• Quenching of light due to presence of stain in gel.	• Elute dye with ethanol.
• Activity levels of isotope too low	• Check calculations.
• Exposure too short for levels of activity used	• Increase exposure time (see *Chapter 4 Guide to exposure times with Amplify, on page 6*).

Problem: Patchy image

Cause	Remedy
• Poor contact between object/subject and film.	• Use good quality cassette that maintains an even pressure over all film surface.
• Impregnation with fluorographic agent not complete.	• Follow Amplify instructions. Allow time for penetration of fluorographic agent especially with thick (≥1 mm) gels.
• Dirt/dust on intensifying screen.	• Keep screens clean.

Problem: Poor resolution

Cause	Remedy
• Poor contact between gel/ subject and film.	• Use good quality cassette.
• Ice crystals formed in wet gels.	• Dry gel before exposure or pre-flash film and use at room temperature.
• Diffusion of bands. Immersion in scintillator too long.	• Follow instructions for use of Amplify.
• Resolution may be lost through use of an intensifying screen. A second beyond the filter paper backing of a gel will transmit light back through the paper further decreasing resolution.	• The use of intensifying screens enhances sensitivity. There is always some loss of resolution incurred in their use.
• Urea in the gel.	• Remove urea by soaking gel in 7% acetic acid.
• Scintillator concentration too high.	• Use Amplify or similar fluorographic reagent.
• Poor initial separation.	• Repeat separation stage. Use freshacrylamide stocks if undertaking SDS PAGE.
• Inherent characteristic of the isotope being used. The more energetic β particles from say 32P can be detected away from the initial localisation of the analyte on the gel.	• Use isotope with lower energy emissions.

Table C. General cloudiness/blackening obscuring the developed image (fogging)

Problem: Fogging of the film only on or near the gel often following the gel outline.

Cause	Remedy
• Contamination of gel components, gel fix or fluorographic reagent with radioactive material.	• Avoid contamination. Count samples of all reagents and discard any containing detectable activity.
• Fluoroescence (Light emission from fluor in substrate)	Dark adapt the gel for at least 30 minutes before exposure to film.
• Static electricity	• See *Table A. Sharply defined images, on page 8*.

Problem: Fogging or artefactual blackening other than on or near the gel.

Cause	Remedy
• Radioactive contamination of the cassette from previous use.	• Clean cassette before use.
• Chemical contamination of cassette. 'Chemography'.	• Clean cassette before use.
• Film in contact with processing chemicals before exposure, especially photographic fixer.	• Clean up all dark-room spills immediately.
• Pressure marks from rollers on automatic processing equipment when used on film removed from cassette at -70°C.	• Allow film to equilibrate to room temperature before processing.

Problem: Even, general fogging

Cause	Remedy
• Safelight used may be at wrong wattage with wrong filter, or too close to working area.	• Check these and change filter/bulb if necessary.
• Light leaking into the darkroom especially if film is left outside lightproof boxes for any length of time.	• Ensure dark room is light proof.
• Old processing chemicals especially exhausted fixer.	• Replace processing chemicals. Check clearing time of a scrap of the emulsion with the fixer being used.
• High radiation background.	• Shield film stocks or move them away from sources of radiation.
• Old film stock passed its expiry date.	• Use fresh film stocks.
• Pre-flash too intense for fluorography.	• Determine experimentally degree of flash required. Use Wratten filters No. 21 or 22 and vary flash distance from film.

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For local office contact information, visit cytiva.com/contact

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