

very hot	>2000 cps on a minimonitor
hot	500-2000 cps on a minimonitor
cool	50-500 cps on a minimonitor

Use a fiber-tipped pen to apply ink of the desired hotness to the pieces of tape.

- When the ink is dry, wrap the sample and backing sheet in Saran Wrap. This prevents contamination of intensifying screens and holders and prevents the sample from sticking to the film.
- In a darkroom, place the sample in an X-ray film holder and cover it with a sheet of X-ray film. Tape the sample and film securely in place. Expose for several hours to several days. Between 1000 cpm and 5000 cpm of ^{32}P in a band 1 cm in width produces an image after an exposure of 12-16 hours.

The most versatile film is Kodak X-Omat AR, which has a high-speed, short-exposure emulsion coated onto both sites of a colorless backing. It may be developed either in an automatic X-ray film processor or by hand:

Kodak liquid X-ray developer	5 minutes
3% acetic acid stop bath or water bath	1 minute
Kodak rapid fixer	10 minutes
running water	15 minutes

Dry in a warm cabinet or at room temperature.

Note. The sensitivity of the film may be increased by the use of intensifying screens (Swanstrom and Shank 1978). The best film-screen combination currently available, two calcium-tungstate-phosphor screens (Dupont Cronex Lightning-Plus) with Kodak X-Omat AR film exposed at -70°C , increases the sensitivity of detection of ^{32}P 8-fold to 10-fold. If only one screen is used, the enhancement factor for ^{32}P is 4-fold to 5-fold. The screens and films should be arranged as shown in Figure A.3. Note that the shiny side of both screens faces toward the film.

In this system, as in conventional autoradiography, the vast majority of events recorded by the film are long-wavelength photons resulting from the fluorescence that occurs when the radiation emitted by the decay of a ^{32}P atom strikes the screen (Laskey and Mills 1977). The response of film to low intensities of light is extremely nonlinear, and the exposure is therefore carried out at -70°C in order to prolong the period of fluorescence. Preexposure (flashing) of the film (Laskey and Mills 1977) does not increase the sensitivity of detection of ^{32}P when using calcium-tungstate screens at -70°C .

- a. The film holder containing the sample, film, and screens is wrapped in aluminum foil and placed in a -70°C freezer for a suitable period of time. Aluminum shields should be interleaved between film holders to prevent exposure to radiation emitted by other samples. A weight should be placed on top of the stack of film holders to ensure that the samples are pressed tightly to the film. If this is not done, blurry, out-of-focus images may result.
- b. The holder is removed from the freezer (use gloves) and the film is removed as quickly as possible and developed immediately. This prevents condensation on the film.
- c. If it is necessary to obtain another autoradiograph, apply another film immediately and return the film holder and screens to the freezer as fast as possible. If condensation forms before you have time to apply a new film, allow the sample and screens to reach room temperature and wipe away all condensation before applying a new film.

BUFFERS FOR RESTRICTION ENDONUCLEASE DIGESTION

Low-salt Buffer

10 mM Tris · Cl (pH 7.5)
10 mM MgCl₂
1 mM dithiothreitol

Medium-salt Buffer

50 mM NaCl
10 mM Tris · Cl (pH 7.5)
10 mM MgCl₂
1 mM dithiothreitol

High-salt Buffer

100 mM NaCl
50 mM Tris · Cl (pH 7.5)
10 mM MgCl₂
1 mM dithiothreitol

Buffer for *Sma*I

20 mM KCl
10 mM Tris · Cl (pH 8.0)
10 mM MgCl₂
1 mM dithiothreitol