U.S. Corporate Headquarters
400 Valley Rd.
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
1(800)343-3291 fax
info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3
D-69214 Eppelheim, Germany
+(49) 6221-765767
+(49) 6221-764620 fax
info@polysciences.de

Polysciences Asia-Pacific, Inc.
2F-1, 207 Dunhua N. Rd.
Taipei, Taiwan 10595
(886) 2 8712 0600
(886) 2 8712 2677 fax
info@polysciences.tw

Ilford Nuclear Materials

The information below is abstracted from Ilford's brochure, Nuclear Research Materials (now out of print). Please use in conjunction with Polysciences' Data Sheet #015.

PRODUCT USE:

Safelight recommendations:
Ilford Nuclear Research emulsions are sensitive to blue light. For general darkroom illumination, the Ilford 902 (light brown) safelight filter in a darkroom lamp fitted with a 15 watt bulb, is recommended. For direct illumination, the Ilford 904 (dark brown) safelight filter is recommended. When maximum illumination is required, a sodium lamp with the correct safelight filters may be used. The safety of this type of lamp should be checked by a practical test before use.

Ilford Nuclear Research emulsion in gel form should not be exposed to safelighting for any longer than necessary. If preparations are to be activated before processing, great care should be taken to avoid prolonged exposure to safelight.

CHECKING THE EMULSION BEFORE USE:

It is possible that Ilford Nuclear Research emulsion may have been exposed in transit to conditions causing the shreds to melt slightly and form a solid lump. These conditions may not have affected the performance of the emulsion. This should be tested before commencing an experiment. To do this, coat a slide in the normal manner (as described below) and process immediately. If the level of background is acceptable and the distribution of developed grains is uniform, the emulsion is undamaged and fit to use. The most common use of this form of nuclear emulsion is for autoradiography of plant and animal material, when a very thin layer of emulsion is coated onto a prepared specimen. This section is largely devoted to this application.

PREPARATION OF SPECIMEN:

When preparing a specimen for autoradiography, it is important to use a histological fixative which will retain the radioactive compound in the tissue during subsequent dehydrating and embedding procedures, and will not affect the sensitivity of the emulsion. Wash the slides on which sections are to be mounted in acid and rinse many times in distilled water. If required, the slides may be subbed to provide a good adhesive surface between the section and the emulsion.

A suitable solution, into which prepared slides may be dipped, is given as follows:

Gelatin	5.0g
Chrome alum ($K_2SO_4 \cdot Cr(SO_4)_3 \cdot 24H_2O$	0.5g
Distilled Water	to 1 litre

Make up the solution just before use. After dipping, drain the slides and dry in a dust free atmosphere.

To avoid chemographic effects (chemicals leaching out of the section into the emulsion and affecting it independently of the nuclear decay), the slide may be dipped in celloidin solution. This procedure is not recommended when low energy isotopes such as H^3 and I^{125} are to be detected. In general, it is better to stain biological specimens before application of the nuclear emulsion. It is important that the chosen stain does not produce chemographic effects.

Coating a slide for visual examination or LM:
Ideally, the environmental conditions in the darkroom are about 25°C and 75%RH.

It has been reported that heating the emulsion before exposure will increase its sensitivity. However, the temperature to which it is heated is critical, because after a certain point, which varies according to the emulsion, the corresponding increase in fog will overshadow any benefits of increased sensitivity.

Remove enough emulsion from the bottle for immediate requirements only. Melt in a glass or stainless steel vessel in a water bath at about 40°C, stirring gently to avoid local overheating. Do not agitate sufficiently to produce froth; if frothing does occur, larger air bubble can be removed by filtering. Ideally, the emulsion should be allowed to melt undisturbed for an hour in the water bath. This has the advantage of reducing the background in the coated slides. Emulsion may be reheated after it has set, but contaminated emulsion must not be returned to the stock bottle.

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For most autoradiographic work, a very thin layer of emulsion is required. This can be obtained by allowing the emulsion to drain off the glass as completely as possible. Emulsion may be applied to the plate either by dipping the slide into the emulsion or by allowing a few drops of emulsion to fall onto the slide. The thickness of the layer when dry will be 1/8 of its thickness at this stage. For example, 0.8ml/dm2 will produce a layer 10µm thick when dry. Hold the slide vertically on a gauze pad for draining and place horizontally for setting and drying.

Very thin layers of emulsion are particularly easy to achieve with K.5D. The thickness of the layer of this emulsion will be 1/16 of its thickness wet. When a slide is dipped into melted K.5D emulsion, the layer that is formed will dry to 3-4µm.

It is important to produce a uniform layer of emulsion, particularly when using an isotope such as C14, which emits high energy b-particles which will penetrate the emulsion. If the coating is not even, particles may be stopped within the emulsion in thick areas and pass right through in thin areas, giving inconsistent results.

Allow the emulsion to set in the dark, then dry it with a gentle current of clean air. The temperature and humidity of the air current are not critical, but drying will be achieved rapidly and safely at 27-30°C. Alternatively, allow the emulsion to dry on a cold metal plate. This will slow down drying, but increase the gelling speed. Slides may also be dried in a carbon atmosphere.

Thinner layers of emulsion can be produced by diluting the emulsion with an equal volume of distilled water, or by the addition of glycerol, which has the effect of reducing any fog resulting from stress between the gelatin and the silver halide crystals. In certain circumstances, it may be better to dilute the emulsion with gelatin. This has the effect of decreasing signal and background. This can be an advantage when examining a specimen with very high activity. In addition, emulsion diluted with gelatin are more sensitive than undiluted one (in relation to their dilution).

Pressure marks are caused by rapid drying or brushing of the plate before it is completely dry.

COATING A PREPARATION FOR ELECTRON MICROSCOPY:

A very thin layer of emulsion is obviously necessary for EM, but little useful information can be derived from a preparation thinner than 3µm.

There are two main methods of coating Ilford Nuclear Research emulsions for EM; the flat substrate method and the loop method. Nuclear emulsion in gel form should never be applied directly to specimens mounted on grids as a highly uneven surface is formed. The flat substrate method is virtually the same as the technique described above. Coat the slide with collodion or formvar-carbon before dipping in the emulsion. Dilute the emulsion with distilled water to ensure an even, thin layer.

The loop method involves dipping a wire loop into melted emulsion that has been diluted with distilled water, to form a membrane. This is applied to a specimen mounted on a grid and left for the required exposure time.

Specimens to be coated by this technique must first be sectioned and mounted on collodion-coated grids, which preferably should be gilded. The sections should be stained and coated with a thin layer of carbon. The specimens are then mounted on small corks, with double-sided adhesive tape. The tape has a hole punched in it, which is slightly smaller than the grids. This apparatus allows the best area of any emulsion membrane to be used each time.

While the usual recommendation for handling Ilford Nuclear Research emulsion in gel form is about 25°C, with this technique it is easier to handle it at about 18°C. Melt the emulsion and allow it to semi-gel. Test the state of gelling at intervals; it should have occurred in 20 minutes. When the emulsion has achieved the correct state, dip a platinum or nickel/chrome wire loop into the emulsion so that the loop is completely covered. Withdraw the loop edge first. Inspect the emulsion membrane in front of a safelight for any imperfections. Allow the membrane to dry in the loop. This takes about 2-3 minutes. Chose the best area and apply it to the specimen. In some circumstances, it may be necessary to add a surfactant to the melted emulsion to ensure a regular monocrystalline layer.

Insert the coated specimens into narrow glass tubes and leave in a light-tight box for the required exposure time.

Exposure:
It is not possible to give general exposure recommenda- tions for Ilford Nuclear Research emulsions.

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The exposure time required for autoradiographic preparations will vary according to the specific activity of the isotope employed, the amount of compound incorporated and its distribution in the material. It is therefore advisable to set up several preparations so that samples can be taken at different intervals of exposure e.g. 2 days, 1 week, 3 weeks, 3-8 months. As a rough guide for biological autoradiographs, a content of 1 microcurie per gram of tissue would require about 20 days exposure, and a preparation for EM would require about 10 times more exposure than a light microscope preparation from the same block of tissue.

PROCESSING:

The method of processing Ilford Nuclear Research materials is largely determined by the thickness of the emulsion layer. It is usual to select an emulsion with the required sensitivity before commencing an experiment, and to develop it fully. In some circumstances, however, partial development may be required. Development is then controlled by longer development in a less active developer. Altering the pH of the developer will also vary the speed of the reaction.

A number of processing techniques are described below. These are intended as a guide and may be modified to suit individual wo rking conditions and experimental aims. During the course of an experimental program, processing techniques should be standardized.

When processing emulsion layers thicker than 50µm, specialized techniques are required: consult the appropriate literature on the subject for further information.

PRE-DEVELOPMENT:

In certain circumstances, it may be necessary to treat the preparation before development. Activation may be achieved by exposure to low levels of light and various chemical intensification methods. In particular, gold latentsification is commonly employed to overcome the loss of sensitivity caused by using a fine grain developer. Pre-development treatment of the preparation with gold thiocyanate gives rise to a 3-4 fold increase in sensitivity.

DEVELOPMENT:

The processing of the thin layers found in autoradiography can be achieved with any standard high energy developer, e.g. an X-ray or paper developer. The exact development time required for an individual experiment is best determined by a development time series. The development time to be used is the shortest time which will achieve complete development of all the latent image centers. Batches of plates and emulsion will vary slightly, so also determine the development time for each new batch by trial.

If an extra fine grain developer is required for EM work, an ascorbic acid-metol developer may be used. These developers are physical developers and give different shaped grain structure after processing than that given with a standard developer. Reduction of signal-to-noise ratios may be achieved by further diluting a standard developer and increasing the development time. This is particularly useful when estimating the number of silver grains by reflectance.

An alternative developer, also suitable for slightly thicker emulsions and autoradiographs for EM is Kodak D-19. For plates up to 50 µm thick, dilute D-19 1+1 with distilled water. Develop autoradiographs for EM in stock solution D-19 for about 2 minutes at 20°C, after which time development should be complete.

FIXATION:

After development, transfer the material to an acid stop bath. Just rinse autoradiographs for EM in distilled water.

The emulsion should then be fixed. Fix the material for twice the time it takes the emulsion to clear. Rapid or hardening fixers are not recommended because of the difficulty of ensuring an efficient wash. Thin preparations take about 2 minutes to fix.

WASHING:

After fixation, thin emulsions may be exposed to normal room light. The required washing time will depend on the thickness of the emulsion, and will be about 15-120 minutes. Slides may be washed in tap water, but give a final rinse in distilled water. While not essential, it is recommended that the wash water temperature is about the same as the temperature of the processing solutions.

STORAGE OF PROCESSED MATERIALS:

After nuclear emulsions have been processed, the storage conditions are less critical than before. If plates and slides are properly processed, with adequate fixing and washing, and are correctly stored, they will keep in good condition for many years. Store processed material that is to be kept for a long time at about 10°C and 50%RH. Variations in temperature and humidity should be kept to a minimum. Store preparations in the dark while they are not being examined.

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STAINING BIOLOGICAL AUTORADIOGRAPHS AFTER PROCESSING:

Tissue sections may be stained through the emulsion after all the residual fixer solution has been removed from the emulsion. The post processing stain must not color the gelatin of the emulsion. The stain should not contrast with nor obscure the developed silver grains of the emulsion. It is important that the staining solutions should not remove developed silver grains from the emulsion and that the stain should not fade.

For studying autoradiographs using the light microscope, toluidine blue is a suitable stain. For studies using the electron microscope, the most widely used stains are alkaline lead stains. These however, are said to cause partial removal of the gelatin. Some workers have intentionally removed the gelatin before staining to give clearer results.

ORDERING INFORMATION:

Ilford Nuclear Emulsions are shipped regularly from Britain directly to Polysciences. Stock is rotated monthly so that you receive fresh material. Emulsions are shipped for next day delivery by UPS. Please refrigerate promptly.

Cat. #	Description	Size
02746	K.5 Emulsion	100 cc
		50 cc
02747	L.4 Emulsion	50 cc
02757	K.2 Emulsion	100 cc
		50 cc
17537	K.5D Emulsion	100 cc

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