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L.R. Gold Resin *Cat.# 17412*
Benzil (UV Catalyst for L.R. Gold) *Cat.# 01946-25*
Benzoin Methyl Ether (UV Catalyst for L.R. Gold) *Cat.# 00425-10*
L.R. White Accelerator *Cat.# 17413*

Introduction:

L.R. Gold is designed for improved morphological definition and immunocytochemical studies at both light and electron microscope levels. LR Gold is a blend of acrylic and hydrophilic monomers. The low viscosity allows specimens to be easily infiltrated for superior sections. Lipid extraction from the tissue and degradation in the electron beam are minimal.

Sectioning at the sub-micron level for electron microscopy to 5 microns for light microscopy can be completed with diamond, glass or tungsten carbide knives. Routine histological stains penetrate very well with some time adjustments. Larger blocks can be trimmed down for electron microscopy allowing thin sectioning and appropriate staining. L.R. Gold can be used directly from alcohols eliminating the need for propylene oxide in the dehydration series.

L.R. Gold can be cured by ultraviolet light in the cold, at 60°C or at room temperature. Cold curing will prevent any exothermic heat generation during the polymerization process. All other procedures will have an exothermic reaction.

Fixation:

L.R. Gold can be used with unfixed or lightly aldehyde fixed tissue specimens. Small specimens of unfixed tissue (maximum size of 3mm X 3mm X 3mm) should be processed in tightly capped vials, at sub-zero temperatures of 0°C to -25°C for all steps. The dehydration and infiltration solutions should be pre-cooled to the appropriate temperature prior to beginning the procedure. This will prevent autolysis during processing and embedding steps.

Well-fixed tissues can be dehydrated, infiltrated at room temperature or 4°C. The final embedding can be room temperature, thermal or on ice as directed.

Specimens requiring fixation can use Poly/LEM Fixative (Cat. #16864) for combination blocks used for both Light Microscopy (LM) and Electron Microscopy (EM). It is 10% Neutral Buffered Formalin with no methanol added. EM fixation with freshly prepared 3 - 4% paraformaldehyde or EM Grade Methanol Free Formaldehyde in PBS at pH 7.2 with or witout 2 1/2% sucrose maintains the cellular integrity and staining intensity for both types of microscopy. Glutaraldehyde - formaldehyde mixtures often cause light or patchy staining with hematoxylin and eosin stains for LM. Standard buffered formalin fixation is often poor for EM structural studies.

Osmium tetroxide fixation should be avoided for dual use blocks as it will interfere or prevent routine LM staining. The first absolute ethanol dehydration step can be completed with 1% phosphotungstic acid (w/v) added to improve electron density under the beam while not interfering with LM staining. If this does not give enough contrast, the ultra thin sections can be post fixed and stained with 1% osmium tetroxide followed by a brief rinse. Exposure to osmium tetroxide vapour on a copper grid under a hood will also postfix the section. Osmium tetroxide must always be used under a hood, with gloves and appropriate protection from fumes. Lead acetate and/or uranyl acetate staining will also increase contrast for viewing.

Dehydration and Infiltration for Low Temperature Processing and Embedding:

Polyvinylpyrrolidone (PVP) is used to protect unfixed tissue during the sub-freezing dehydration series. The PVP at either 10,000MW or 40,000MW is dissolved in either methyl or ethyl alcohols, water as the diluent and L.R. Gold resin. All dilutions are pre-mixed and cooled to the recommended temperatures prior to use. The final infiltration steps (8 and 9) should be completed in BEEM capsules or appropriate embedding mold. The following dehydration schedule is to be used for unfixed specimens:

1.	50% Absolute alcohol/50% DI water with 20% PVP	0°C	15 Minutes
2.	70% Absolute alcohol/30% DI water with 20% PVP	-25°C	45 Minutes
3.	90% Absolute alcohol/10% DI water with 20% PVP	-25°C	45 Minutes
4.	60% L.R. Gold Resin/40% Absolute Alcohol/10% PVP	-25°C	30 Minutes
5.	70% L.R. Gold Resin/30% Absolute Alcohol/10% PVP	-25°C	60 Minutes
6.	100% L.R. Gold Resin/10% PVP	-25°C	60 Minutes
7.	100% L.R. Gold Resin with Initiator*	-25°C	60 Minutes
8.	100% L.R. Gold Resin with Initiator*	-25°C	Overnight
9.	100% L.R. Gold Resin with Initiator*	-25°C	24 Hours

* The initiator, Benzil (UV Catalyst) is mixed as 0.01gm in 10mL of L.R. Gold Resin until dissolved. PVP is not used with resin containing initiator.

Dehydration and Infiltration for Room Temperature /Thermal Processing and Embedding:

Standard dehydration times for the specimen in graded alcohols should be completed. Acetone is not recommended for use with L.R. Gold as it acts as a free radical scavenger and inhibits the polymerization process.

See Fixation section for fixatives. Specimens used in this protocol should be well fixed. The suggested dehydration times are for specimens at 1mmX1mmX1mm. Larger samples will require more time in the solutions. The specimen is transferred from the fixative to the dehydration series as follows:

1.	70% Ethanol/DI water	RT	15 Minutes
2.	70% Ethanol/DI water	RT	15 Minutes
3.	80% Ethanol/DI water	RT	15 Minutes
4.	95% Ethanol/DI water	RT	15 Minutes
5.	95% Ethanol/DI water	RT	15 Minutes
6.	100% Ethanol/DI water	RT	15 Minutes
7.	100% Ethanol/DI water	RT	15 Minutes
8.	L.R. Gold Resin	RT	30 Minutes
9.	L.R. Gold Resin	RT	1 Hour or until the tissue sinks and becomes translucent
10.	L.R. Gold Resin with initiator (See * above)	RT	30 Minutes

Embedding:

Prepare the embedding solution by adding 0.01gm of Benzil (UV Catalyst) or 0.05gm Benzoin Methyl Ether (for cold polymerization) to 10mL of L.R. Gold Resin and mix until dissolved. The amount of solution can be increased as needed. However, only the amount required for embedding should be prepared.

The tissue should be removed and placed in a BEEM capsule or appropriate mold for UV, thermal or cold polymerization. The embedding resin mixture can be pipetted into the mold and the specimen reoriented if needed prior to positioning over the UV lamp source or in the oven for curing.

The mold should be a clear to semi-transparent High Density Polyethylene (HDPE) which will allow light to penetrate evenly and easily for UV curing. A holder that can be placed above the light source at approximately 20cm should support the mold.

Several types of light source can be used for polymerization. A quartz-halogen bulb is placed 20cm from the bottom of the mold to be polymerized and turned on for several hours to overnight. The block should polymerize evenly at this distance.

Room temperature UV polymerization can be completed by adding 0.1% benzoyl peroxide to 10mL of L.R. Gold Resin and dissolving just prior to embedding. The mixture will begin to polymerize in approximately 5 minutes. The polymerization can be done on ice to reduce the polymerization time slightly and reduce exothermic reaction. Although the polymerization reaction is very rapid the curing time can be hastened by adding L.R. White accelerator at 20µl per 20mL of resin with benzoyl peroxide just prior to embedding. This mixture will polymerize very quickly and have a higher exothermic reaction.

Sectioning and Mounting

L.R. Gold will require a microtome that is designed to section plastics for LM or an ultramicrotome for EM. Routine paraffin microtomes are not appropriate for plastics, as they will cause chatter in the sections. The use of tungsten carbide triangular knives for small blocks can replace glass knives. Larger blocks will require either the larger tungsten carbide blade or glass Ralph knives. Smaller block faces can be sectioned as thin as 0.25µ with either knife type or a diamond knife for ultra thin sections for EM. Larger blocks will be sectioned at 1.0µ to 5µ with the tungsten carbide blades. Thicker sections may become more difficult to section with chatter or shattering of the material.

Sections for EM can be picked up on grids as usual. The sections should be allowed to dry and then stained with appropriate heavy metal reactions. Uranyl acetate and lead citrate are the most common to enhance density for viewing.

Mounting can be done on glass slides with a drop of water to help expand the plastic. An applicator stick soaked in acetone gently waved over the section and water will further expand the section. The slide can be placed on a hot plate at 60°C to 70°C to evaporate the water and adhere the section to the slide. A solution of 30% to 40% acetone and water can used to float the sections followed by air drying prior to placement on the hot plate. **DO NOT USE ACETONE ON THE HOT PLATE.**

Staining

LM sections will stain with most routine stains at 2µ and thicker. Some extension of times may be required to allow penetration of the stains. Solutions made in ethanol or methanol may result in softening the surrounding L.R. Gold in the section and lifting it from the slide. Alcohol based stains should be used with the shortest possible times or an aqueous base stain used where possible. Dehydration through alcohols should be avoided or shortened to very quick dips. Sections, which do not require alcohol or have been quickly dipped can be blotted and/or allowed to air dry to prevent sections from falling off the slide.

Multiple Stain Solution (MMS) is an excellent locational stain for very thin sections to assess the block face or when Hematoxylin and Eosin Y are just to harsh for the sections causing them to float off. This is a quick stain and is used by either flooding the slide for 15 to 30 seconds or submerging and staining with a 2% to 3% aqueous MMS solution for 1 to 3 minutes followed by a rinse to remove the excess stain. Allow to air dry and coverslip to view.

Electron microscopy staining with lead citrate, uranyl acetate, and other heavy metals can be used as routine protocols.

Storage of L.R. Gold Resin and Accelerator:

L.R. Gold Resin should be stored at 4°C when not in use. The cap should be very tight to prevent condensation and oxygen during periods at room temperature. The UV catalyst can be stored in a cool dark area at room temperature.

Safety and Handling Precautions:

Causes eye and skin irritation. L.R. Gold should be used under a hood. Gloves should be worn when handling the components for measuring and dispensing.

Ordering Information:

Catalog #	Description	Size
17412-500	L.R. Gold	500mL
17413-10	L.R. White Accelerator	10mL
00425-10	Benzoin Methyl Ether (Cold Polymerization)	10gm
01946-25	Benzil (Photo Polymerization Catalyst)	25gm
01052-250	Poly (N-vinylpyrrolidone)	250gm
17411-500	L.R. White	500mL
04018-1	10% Formaldehyde, Ultra Pure EM Grade Methanol Free	1L
04018-4	10% Formaldehyde, Ultra Pure EM Grade Methanol Free	4X1L
18814-20	16% Formaldehyde, Ultra Pure EM Grade Methanol Free	20X10mL
07710-100	Glutaraldehyde, EM Grade 8%	100mL
07710-5	Glutaraldehyde, EM Grade 8%	5X100mL
0216A-10	Glutaraldehyde, EM Grade 8%, Ampoules	10X10mL
00216-30	Glutaraldehyde, EM Grade 8%, Ampoules	30X10mL
01909-100	Glutaraldehyde, EM Grade 25%	100mL
01909-5	Glutaraldehyde, EM Grade 25%	5X100mL
01909-10	Glutaraldehyde, EM Grade 25%, Ampoules	10X10mL
18428-100	Glutaraldehyde, EM Grade 50%	100mL
18428-5	Glutaraldehyde, EM Grade 50%	5X100mL
18428-10	Glutaraldehyde, EM Grade 50%, Ampoules	10X10mL
01201-10	Glutaraldehyde, EM Grade 70%, Ampoules	10X2mL
01201-5	Glutaraldehyde, EM Grade 70%, Ampoules	5X10mL
00380-1	Paraformaldehyde EM Grade	1Kg
00380-250	Paraformaldehyde EM Grade	250gm
0223A-5	Osmium Tetroxide Crystalline 99.95% Pure	5X1gm
0223B-10	Osmium Tetroxide Crystalline 99.95% Pure	10X1gm
0023C-10	Osmium Tetroxide Crystalline 99.95% Pure	10X1/2gm
0223D-10	Osmium Tetroxide Crystalline 99.95% Pure	10X1/4gm
0972A-20	Osmium Tetroxide 4% Solution	20X2mL
0972B-5	Osmium Tetroxide 4% Solution	5X10mL
0972C-20	Osmium Tetroxide 4% Solution	20X10mL
23310-10	Osmium Tetroxide 2% Solution	10X2mL
23311-10	Osmium Tetroxide 2% Solution	10X5mL
16864-3.75	Poly/LEM Fixative (10% NBF not stabilized for LM & EM)	3.75L
09860-1	Reagent Alcohol	1Gal
24216-1	Tissue Tack Silane Charged Microscope Slides	1 Box
22247-1	Poly L Lysine Coated Microscope Slides	1 Box
18985-1	Peel-A-Way® Embedding Mold Truncated, 8mm bottom 22mm square top	288 ea./case
18986-1	Peel-A-Way® Embedding Mold Truncated, 12mm bottom 22mm square top	288 ea./case
18646A-1	Peel-A-Way® Embedding Mold, 22x22mm, 20mm deep	288 ea./case
18646B-1	Peel-A-Way® Embedding Mold, 22x30mm, 20mm deep	288 ea./case
18646C-1	Peel-A-Way® Embedding Mold, 22x40mm, 20mm deep	264 ea./case
18646D-1	Peel-A-Way® Disposable Embedding Molds Sampler Pack	1 package

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