

Microscopy

SERVA
Electrophoresis

Reagents for Light and Electron Microscopy

Light Microscopy

Dyes and Stains

Embedding & Mounting Media

Other Reagents

Electron Microscopy

Fixation

Dehydration

Embedding

Staining and Contrasting

Application Note

Content

Light Microscopy

1. Introduction	3
2. Dyes and Stains	3
3. Embedding & Mounting Media	5
4. Other Reagents for Microscopy	6
5. References	9

Electron Microscopy

1. Introduction	10
2. Fixation	10
3. Dehydration	12
4. Embedding	13
5. Staining and Contrasting	17
6. Application Note: Specimen Preparation and Embedding of Rodent Brain Tissue	18
7. References	19

Light Microscopy

1. Introduction

Study of biological specimens by microscopy requires several steps of sample preparation like fixation, dehydration, embedding, cutting, staining, and mounting. For over 50 years SERVA offers a comprehensive range of dyes and reagents for these manipulations. The most important aspect is to ensure the reproducibility of the results. SERVA's stringent control guarantees highest quality and reliability of all products.

2. Dyes and Stains

Histological and cytological investigations rely on a variety of stains. Different stains are used to visualize cell and tissue components in animal and plant material. The dyes are furthermore used in microbiology to make micro-

organisms visible, differentiate them due to distinct staining properties or determine cell viability. SERVA offers high quality dyes and staining kits for applications in botany, bacteriology, histology, and cell culture.

Description	Application	Size	Cat.No.
Acriflavine HCl	Fluorochrome and fluorochrome Schiff reagent, e.g. in Kasten's fluorescent PAS method (22, 23, 24, 25)	25 g	10671.01
		100 g	10671.02
Alcian Blue 8 GS (8GX)	Staining of glycoproteins and glycosaminoglycans (5, 6, 21, 34, 37, 48, 49)	10 g	12021.01
		50 g	12021.02
Aniline Blue	Contrast stain in histology and cytology, e.g. for the detection of Callose in plant and staining of various types of connective tissue, pollen grains, fungus spores, and others (5, 6)	25 g	13645.01
Azorubin	For staining of collagen fibers (6)	25 g	14410.01
Bromophenol Blue • Na-salt	Indicator pH 3.4 (yellow) – 4.6 (purple), used for vital staining of sperm	5 g	15375.01
		25 g	15375.02
Carmine	Staining of nuclei (Orth's Lithium Carmine), glycogen (according to Best), chromosomes (as iron acetocarmine), and pollen tubes (5, 6)	5 g	16180.01
		25 g	16180.02
Congo Red	Indicator pH 3.0 (blue) – 5.2 (reddish orange); contrast stain to Hematoxylin, for plant mucin and cellulose, negative staining of bacterial capsules, specific stain for amyloids in pathology (5, 6, 26, 38)	25 g	27215.01
Crystal Violet	Indicator in the acid pH scale: 0.0 (violet-blue) – 1.8 (yellow); Gram-positive stain in bacteriology, staining of amyloids, vascular bundles, nuclei and chromatin, differentiation of lignified and non-lignified cell walls (with Erythrosin B) (2, 5, 6)	25 g	27335.01
		100 g	27335.02
3,3'-Diaminobenzidine • 4HCl (DAB)	For staining in histology and demonstration of ultrastructural peroxidase (16, 19)	100 mg	18865.01
		1 g	18865.02
4',6-Diamidino-2-phenylindole • 2HCl (DAPI)	For demonstration of mycoplasmas and viruses in cells and fluorescent chromosome staining (43, 45)	10 mg	18860.01
		25 mg	18860.02
1,9-Dimethyl-methylene blue • chloride	Histological stain with strong metachromic properties; for quantification and discrimination of sulfated glycosaminoglycans (10, 28, 46, 47)	1 g	20335.01

Description	Application	Size	Cat.No.
Eosin Y•Na-salt	Fluorescent indicator; counter stain for Hematoxylin (e.g. for staining nuclei, erythrocytes or collagen) and component in various Romanowsky stains for hematological studies (5, 6)	25 g	21005.01
Ethidium bromide	Counter stain for cell nuclei in histological and cytological studies and for staining of DNA after gel electrophoresis (5, 6, 32)	1 g	21238.01
		5 g	21238.02
Fast Green FCF	Staining of connective and nervous tissue, muscle, cytoplasm, erythrocytes, nuclei, meristemic and other plant tissues (together with Safranin O); for quantitative evaluation of proteins in polyacrylamide gel electrophoretic separations (1, 3, 5, 6, 13)	5 g	21295.01
		25 g	21295.02
Fast Red TR-salt	Alkaline phosphatase and esterase assays (4, 15, 18)	50 g	21317.01
Fluorescein diacetate	For study of cell membranes, histochemical esterase detection and fluorescent viability stain (17, 41, 42)	5 g	21575.02
Fuchsin acid	Indicator pH 12 - 14 and for histological staining of plasma, tissue and collagen, often in combination with other stains (5, 6)	25 g	34597.01
		100 g	34597.02
Fuchsin basic	Staining of nuclei (Feulgen´s stain for DNA), glycogen, mucin, elastic tissue, fuchsinophil granules and the cell walls of certain bacteria (5, 6)	25 g	21916.02
		100 g	21916.03
Gallein Staining Kit	An alternative to nuclear staining with Hematoxylin, nuclei will be coloured red (6)	1 kit	23321.01
Hematoxylin	Cytological and histological staining, mainly as nuclear stain and in combination with the contrast stain Eosin (Papanicolaou stain) (5, 6)	25 g	24420.01
		100 g	24420.02
Malachite green oxalate	Acid-base indicator: pH 0.0 (yellow) – 2.0 (green) and ph 11.6 (blue-green) – 14.0 (colorless); staining in histology, bacterial spores and in plant pathology with the Pianese technique to study sections of tissue infected by fungi (5, 6, 44)	25 g	28335.03
Methylene Blue	Redox indicator, acting as hydrogen acceptor; staining in bacteriology, parasitology, hematology, and histology: e.g. for staining of blood cells, bacteria in dairy products, Negri bodies in sections (inclusion body found in the cytoplasm of certain nerve cells containing the rabies virus) and nuclear stain; for detecting RNA in gel electrophoresis (5, 6, 20, 30)	25 g	29198.01
		100 g	29198.02
Myrthilin Staining Kit	An alternative to nuclear staining with Hematoxylin; nuclei will be coloured purple	1 kit	23322.01
Neutral Red	Indicator pH 6.8 – 8.0 and for nuclear, supravital, lipid fluorochrome and volutin staining (5, 6, 8, 27)	25 g	30305.01
Parafuchsin	Indicator (pH 1.0 – 3.1) and for staining of DNA according to Feulgen (Schiff´s base) and with the PAS method to stain glycogen, mucin, reticulum, collagen, basement membranes and fibrin; for staining of some intracellular parasites: Rickettsiae, Coxiella, and Chlamydiae (5, 6, 11, 12, 31, 35, 36)	5 g	31627.01
		25 g	31627.02
Ponceau S	Histological staining of connective tissue, fibrin, and thrombocytes (6)	5 g	33429.01
		25 g	33429.02
Safranin O	Counter stain for Gram staining in smears; nuclear stain in plant anatomy with various counter stains: e.g. Fast Green FCF or Aniline Blue for meristemic tissue, Orange G for showing cell walls and protoplasts (2, 5, 6)	10 g	34598.01
Tetranitro-tetrazolium blue•chloride	Cytochemical localization of succinic dehydrogenase (14, 40)	250 mg	35935.02
		1 g	35935.03
Toluidine Blue O salt	Staining for oligodeoxyribonucleotides, RNA, mucopolysaccharides and proteoglycans; for staining colonies of mycoplasma and L-phase variants according to Dienes and of Nissl bodies in nerve cells (5, 6, 7, 9, 29, 33, 39, 50)	25 g	36693.02

3. Embedding and Mounting Media

Embedding agents are used to receive defined hardness and homogeneity of the material to be processed. This is crucial to achieve uniform sectioning quality. SERVA offers a special embedding medium, Histoplast-S, but as well traditional embedding media like gelatin and Kaiser’s Glycerol gelatin.

Permanent specimens in histology/light microscopy are prepared by mounting in non-aqueous or aqueous media depending on the protocol involved. Prep-

arations must be fully dehydrated, if non-aqueous agents are used. The refractive index of the mounting media should be around 1.5, the refractive index of glass.

Many of the traditional mounting media contain hazardous chemicals like xylene or toluol. The Includal mounting medium series is a safe alternative without solvents, phenol or other compounds.

A. Aqueous Media

Description	Application (Refractive Indexes)	Size	Cat.No.
Fluoromount W for microscopy	Non-fluorescent, aqueous mounting medium for microscopy, ideal for F.I.T.C. (1.39 - 1.40)	50 ml	21634.01
Gelatin	Embedding and aqueous mounting medium, coating of cover slips for adherence of tissue culture cells (1.51 - 1.53)	500 g	22151.02
Glycerol	Aqueous mounting medium for microscopy (1.46 - 1.48)	1 L	23176.01
Glycerol albumen	Aqueous mounting medium for microscopy	250 ml	20918.03
Glycerol gelatin for microscopy	Universal aqueous slide mounting medium for microscopy (1.46 - 1.48)	100 g	23310.02
Glycerol gelatin after Kaiser, phenol-free	Universal aqueous slide mounting medium for microscopy; as well suitable for water-soluble embedding (1.46 - 1.48)	50 ml	23311.01
Glycerol gelatin after Kisser, phenol-free	Aqueous slide mounting medium for microscopy, especially suitable for the preparation of pollen (1.46 - 1.48)	15 ml	23312.01
Includal A	Universal aqueous slide mounting medium for microscopy, suitable for all standard staining methods (1.34 - 1.36)	10 ml	23314.01
Includal AC after Hoyer	Aqueous slide mounting medium for microscopy, especially suitable for entomological and botanical specimens (1.37)	30 ml	23315.01
Includal DMHF	Aqueous slide mounting medium for microscopy, especially for use in entomology (1.38 - 1.40)	10 ml	23320.01
Includal PVA	Aqueous slide mounting medium for microscopy, especially suitable for entomological and histological specimens (1.43 - 1.47)	10 ml	23316.01
Includal PVL	Aqueous slide mounting medium for microscopy, especially for use in entomology (1.42 - 1.44)	10 ml	23318.01
Includal PVLA	Aqueous slide mounting medium for microscopy, especially for use in entomology (1.41 - 1.43)	10 ml	23319.01
Includal PVP	Aqueous slide mounting medium for microscopy, especially suitable for sensitive staining (1.42 - 1.44)	10 ml	23317.01
Sorbitol gelatin after Clemens, phenol-free	Aqueous slide mounting medium for microscopy, especially suitable for nuclear staining with Hematoxylin as well as lipid and metachromatic staining (1.44 – 1.47)	15 ml	23313.01

B. Non-aqueous Media

Description	Application (Refractive Indexes)	Size	Cat.No.
Canada balsam for microscopy	Traditional non-aqueous mounting medium (1.520 - 1.525)	100 ml	26896.02
DePeX	Non-aqueous mounting medium for histology (1.52 - 1.53)	100 ml	18243.01
		500 ml	18243.02
Fluoromount for microscopy	Non-fluorescent, non-aqueous mounting medium for microscopy (1.45 - 1.46)	50 ml	21644.01
Histoplast-S	Special embedding medium for histology	1 kg	24895.01
Includal CB for microscopy	Non-aqueous, non-volatile mounting medium for microscopy (1.51)	100 ml	23324.01

4. Other Reagents for Microscopy

A. Immersion Media

Immersion media are liquids with a refractive index about 1.5, comparable to the refractive index of glass. They are used together with immersion slides and are located between the surface of the specimen and the microscope lens.

Description	Application	Size	Cat.No.
Cedar wood oil	Immersion oil for microscopy	50 ml	38565.01
		500 ml	38565.02
Ethylene glycol	Immersion medium for microscopy	1 L	11285.02

B. Decalcifier

Microscopic examination of bone and other hard tissues in histology can only be done after decalcification procedures. Bone and hard tissue requires the use of inorganic acids as in the case of Decal, for liberating the acids of the mineral salts, which then can be rinsed out. For decalcifying sensitive, calcium-containing tissue, SERVA's Decalcifier concentrate is recommended, which contains a chelating agent binding the calcium ions of the tissue. This type of decalcifying agent preserves better the antigen structures in the tissue for subsequent immunological procedures.

Description	Application	Size	Cat.No.
Decal	For the rapid decalcification of histological sections	500 ml	18140.02
Decalcifier concentrate plus indicator	For the rapid decalcification of histological sections	100 ml	18141.01

C. Fixing and Dehydration Reagents

The major goal of fixation of biological samples is the optimal preservation of all natural structures while at the same time effectively preventing processes like autolysis, decomposition, and loss of low- and high-molecular weight substances during the subsequent treatments. Type and size of the material as well as required embedding and staining methods determine which fixing agent is selected.

Since many embedding and mounting media are not water soluble, dehydration of the specimen by stepwise increasing the concentration of a suitable organic solvent is necessary prior to embedding or mounting.

Description	Application	Size	Cat.No.
Ethanol undenatured 96 %	Fixing and dehydration of sections by ascending alcohol series	1 L	11094.01
		2.5 L	11094.02
Ethanol undenatured absolute	Fixing and dehydration of sections by ascending alcohol series	250 ml	11093.01
		1 L	11093.02
		2.5 L	11093.03
Isopropanol	Fixing and dehydration of sections by ascending alcohol series	250 ml	39559.01
		1 L	39559.02
Paraformaldehyde	Fixation of sections	100 g	31628.01
		500 g	31628.02
Propylene oxide	Dehydrating agent	500 ml	33715.03
5-Sulfosalicylic acid	Fixation of sections	100 g	35706.01
Trichloroacetic acid	Fixing agent	100 g	36910.01
		500 g	36910.03

D. Buffers and Other Reagents

SERVA offers a wide range of necessary buffers and reagents for microscopical applications in cytology, histology and bacteriology.

Description	Application	Size	Cat.No.
Boric acid	Addition to stain solutions, buffering substance	250 g	15165.02
		1 kg	15165.01
Calcium chloride x 2H ₂ O	Addition to stain solutions	250 g	39551.01
Citric acid x H ₂ O	Addition to stain solutions, buffering substance	500 g	38640.01
		1 kg	38640.02
		5 kg	38640.03
EDTA·Na ₂ salt	Addition to stain solutions, buffering substance	100 g	11280.01
		1 kg	11280.02
		5 kg	11280.03

Description	Application	Size	Cat.No.
HEPES	Buffering substance	25 g	25245.02
		100 g	25245.03
		250 g	25245.04
		1 kg	25245.05
		5 kg	25245.06
HEPES-Na salt	Buffering substance	100 g	25249.03
		250 g	25249.05
		1 kg	25249.04
MES	Buffering substance	100 g	29834.02
		500 g	29834.04
		1 kg	29834.03
MES, monohydrate	Buffering substance	100 g	29830.01
		250 g	29830.02
		500 g	29830.06
		1 kg	29830.03
MOPS	Buffering substance	100 g	29836.02
		500 g	29836.03
		1 kg	29836.04
PEG 4000	Polyethylene glycol dissolved in lower glycols in place of paraffin wax is used for embedding of histological and other medical specimens.	500 g	33136.01
		5 kg	33136.02
PEG 6000	Polyethylene glycol dissolved in lower glycols in place of paraffin wax is used for embedding of histological and other medical specimens.	500 g	33137.01
		5 kg	33137.02
PIPES	Buffering substance	50 g	32981.01
		250 g	32981.02
Potassium chloride	Buffering substance	1 kg	26868.02
Potassium dihydrogen phosphate, anhydrous	Buffering substance	500 g	26870.01
di-Potassium hydrogen phosphate, anhydrous	Buffering substance	500 g	26887.01
Sodium dihydrogen phosphate x 2H ₂ O	Buffering substance	1 kg	30186.02
di-Sodium hydrogen phosphate x 2H ₂ O	Buffering substance	500 g	30200.01
TRIS	Buffering substance	100 g	37180.02
		500 g	37180.03
		1 kg	37180.05
		2.5 kg	37180.04
TRIS•hydrochloride	Buffering substance	100 g	37192.01
		500 g	37192.02
		2.5 kg	37192.03

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Electron Microscopy

1. Introduction

In electron microscopy, accelerated electrons are used to form an enlarged image of a specimen. To visualize the fine structure of biological material, ultrathin sections are necessary to be penetrated by the electrons. For this purpose, several steps of sample preparation are required.

The major operations are:

Fixation:	For preservation of ultrastructure of cells and protection of specimens against the subsequent manipulations
Dehydration:	Necessary when using water-immiscible resins for embedding
Embedding:	Necessary for preparation of ultra-thin sections
Sectioning:	Ultra-thin sections are necessary to be penetrated by the electron beam
Staining and contrasting:	Visualization of cell structures by using specific heavy metals which cause elastic scattering of electrons

SERVA offers most of the chemicals which are commonly used for these manipulations.

2. Fixation

Fixation is the first step in the preparation of biological specimens for investigation by electron microscopy and is necessary to prevent (5, 7, 8, 11):

- Post-mortal changes of the cell caused by autolysis
- Loss of low- and high-molecular weight substances during the following treatments (dehydration, embedding, staining)
- Fixation results in crosslinking of lipids and proteins
- Possible structural changes due to the further preparation steps and the treatment with heavy metals

The goal is an optimal preservation of all natural structures of the cell or tissue.

The most frequently used fixatives are glutaraldehyde, paraformaldehyde and osmium tetroxide or a mixture of glutaraldehyde and paraformaldehyde. Glutaraldehyde reacts with proteins and causes their cross-linking. It is often used as primary fixative because it is effective in preserving fragile structures (e.g. microtubules, rough and smooth endoplasmic reticulum, mitotic spindles and platelets) better than any other fixative. Paraformaldehyde is better suited as fixative in immunocytochemistry as it allows a better penetration of the antibodies into the fixed tissue than glutaraldehyde fixed material. Osmium tetroxide is normally used after an initial aldehyde fixation. It is supposed that it reacts mainly with lipids. Besides it acts also as an electron stain, a major advantage over most other known fixatives.

To avoid artefacts and changes in the structure of cell components, it is important to observe several parameters: The fixative has to be dissolved in a buffered solution with a pH and an ionic composition close to the conditions in the native state so that materials are neither extracted or precipitated during fixation. The solution should have a suitable osmolarity so that shrinkage or swelling of the cells or the tissue is avoided. The optimal method of fixation depends essentially on the specimen and has to be found by trial and error. To avoid possible reactions between fixative and dehydration agents, the specimens should be rinsed after fixation.

SERVA Reagents for Fixation

Description	Size	Cat. No.
Dimethylsulfoxide	250 ml	20385.01
	1 L	20385.02
Glutaraldehyde 25 % solution in water for electron microscopy, high purity	25 ml	23114.01
	10x 5 ml	23114.02
Glutaraldehyde 25 % solution in water for electron microscopy, standard grade	250 ml	23115.01
	2.5 L	23115.03
Glutaraldehyde 50 % solution in water for electron microscopy	25 ml	23116.01
	10 x 5 ml	23116.02
Osmium tetroxide for electron microscopy	100 mg	31251.01
	500 mg	31251.02
	1 g	31251.03
Osmium tetroxide 4 % solution for electron microscopy	2 ml	31253.01
	10x 2 ml	31253.02
	10 ml	31253.03
	5x 10 ml	31253.04
	100 g	31628.01
Paraformaldehyde	100 g	31628.01
	500 g	31628.02

SERVA Buffer Substances

Description	Size	Cat. No.
Cacodylic acid • Na-salt x 3 H ₂ O	25 g	15540.01
	100 g	15540.02
	500 g	15540.03
HEPES	25 g	25245.02
	100 g	25245.03
	250 g	25245.04
	1 kg	25245.05
	5 kg	25245.06

SERVA Buffer Substances

Description	Size	Cat. No.
HEPES Na-salt	100 g	25249.03
	250 g	25249.05
	1 kg	25249.04
MES, monohydrate	100 g	29830.01
	250 g	29830.02
	500 g	29830.06
	1 kg	29830.03
MES	100 g	29834.02
	500 g	29834.04
	1 kg	29834.03
MOPS	100 g	29836.02
	500 g	29836.03
	1 kg	29836.04
PIPES	50 g	32981.01
	250 g	32981.02
Potassium dihydrogen phosphate, anhydrous	500 g	26870.01
di-Potassium hydrogen phosphate, anhydrous	500 g	26887.01
Sodium dihydrogen phosphate x 2 H ₂ O	1 kg	30186.02
di-Sodium hydrogen phosphate x 2 H ₂ O	500 g	30200.01

3. Dehydration

Most of the embedding media used today are not water soluble. Consequently, the fixed samples have to be dehydrated by passing them through a sequence of solutions, the last of which is miscible with the embedding medium.

In practice, dehydration can be performed by stepwise increasing (e.g. 30 %, 50 %, 70 %, 90 %, 96 %, and several times 100 %) the concentration

of a suitable organic solvent (ethanol, methanol or acetone).

When using ethanol as main dehydration agent, it is necessary to replace it in the last step by propylene oxide, because most resins are not readily miscible with ethanol. When acetone is applied as main dehydration agent, the use of propylene oxide as a transitional solvent is not necessary (5, 8, 11).

SERVA Reagents for Dehydration

Description	Size	Cat. No.
Ethanol undenatured, absolute	250 ml	11093.01
	1 L	11093.02
	2.5 L	11093.03
Ethanol undenatured 96 %	1 L	11094.01
	2.5 L	11094.02
Methanol for HPLC	2.5 L	45630.01
Propylene oxide	500 ml	33715.03

4. Embedding

In the following section, some commonly used embedding media are listed. We refer to the composition of the original reference. Variations can be done in dependence on the specimen under study. Hardness

of a block can be manipulated by changing the relation between monomer and cross linker (hardener) and/or by the addition of plasticisers (1, 5, 8, 11).

A. ERL 4221D, SPURR-Mixture (formerly ERL 4206)

This resin is characterized by its low viscosity which facilitates rapid penetration into tissues. It is often used for highly vacuolated plant cells and tissue with hard

lignified cell walls, for brain tissue as well as for tissues with dense structures like minerals and bones (12).

	Standard	Modification			
Ingredients	A	B	C	D	E
ERL 4221 D	10.0 g	10.0 g	10.0 g	10.0 g	10.0 g
D.E.R. 736	6.0 g	4.0 g	8.0 g	6.0 g	6.0 g
NSA	26.0 g	26.0 g	26.0 g	26.0 g	26.0 g
DMAE	0.4 g	0.4 g	0.4 g	1.0 g	0.2 g
Total weight	42.4 g	40.4 g	44.4 g	43.0 g	42.2 g
Polymerisation time (h) at 70 °C	8	8	8	3	16
Hardness	Firm	Hard	Soft	1)	2)
Pot life (d)	3 - 4	3 - 4	3 - 4	2	7

- 1) Modification D results in shorter pot life, rapid cures and higher viscosity
- 2) Modification E yields longer pot life and lower viscosity

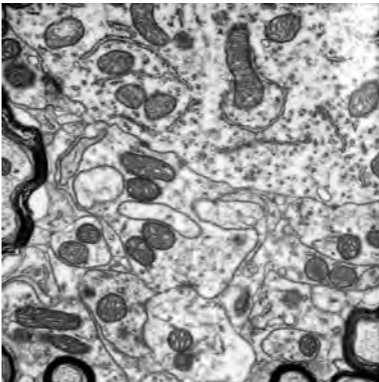


Figure 1: Calyx of Held from rat brain, embedding with ERL-4221 after Spurr, modified, 17.000x magnification, kindly provided by H. Horstmann, Institute of Anatomy, University of Heidelberg

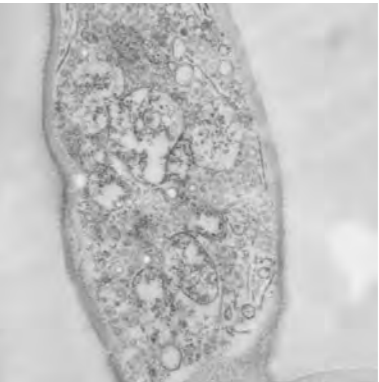


Figure 2: Yeast cell, embedding with ERL-4221 after Spurr, modified, 17.000x magnification, kindly provided by H. Horstmann, Institute of Anatomy, University of Heidelberg

B. Glycid ether 100 (EPON 812)

Glycid ether 100 (formerly known as EPON 812) is one of the most widely used embedding media. Sections of this resin show greater contrast in the electron microscope than that of comparable Araldite sections. The resin shows excellent sectioning quality and is best suited for the preparation of serial sections to obtain three-dimensional information on tissue structure (3). The inherent granularity of glycid ether 100 may however limit high magnification and high resolution.

Glycid ether cured with dodecenylsuccinic acid anhydride (DDSA) alone will result in rather soft blocks, whereas methylnadic anhydride (MNA) yields very hard blocks. The method of Luft uses two different stock solutions A and B. By using varying proportions of the two mixtures, blocks of the desired hardness can be produced. For the preparation of serial sections of brain tissue, the following embedding formulation has been shown to give excellent results (3, 14).

Method of Luft (9)

Mixture A [§]	Mixture B ^{§§}	DMP-30	Relative hardness
10 ml	0 ml	0.15 ml	Very soft
7 ml	3 ml	0.15 ml	Soft
5 ml	5 ml	0.15 ml	Medium
3 ml	7 ml	0.15 ml	Hard
0 ml	10 ml	0.15 ml	Very hard

Polymerisation: 20 h at 45 °C and then at 60 °C for again 24 h or alternatively for 36 - 48 h at 60 °C.

[§] Mixture A

Glycid ether 100	62 ml
DDSA	100 ml

^{§§} Mixture B

Glycid ether 10	100 ml
MNA	89 ml

These mixtures are stable for 6 months, if stored under argon at -20 °C.

Glycid ether 100 resin hard (3,14)

Glycid ether 100	37 g
DDSA	25 g
MNA	20 g
BDMA	1.3 g

Polymerisation: 24 - 72 h at 60 °C

Due to the fact that the epoxide content of glycid ether 100 may vary from lot to lot, it may be difficult to prepare blocks of reproducible hardness. To circumvent this problem, Burke and Geiselman developed a method of calculating the correct proportions

of glycid ether 100, DDSA and MNA to use with any particular batch of resin; they provide a table listing these proportions for any particular WPE (weight per epoxide, epoxide equivalent) (2, 8).

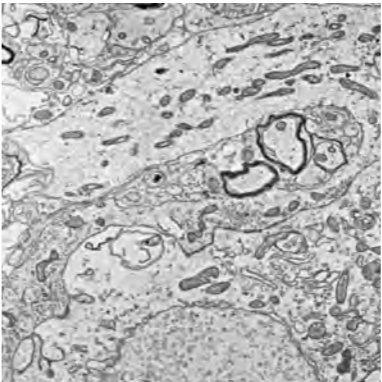


Figure 3: Calyx of Held from rat brain, embedding with Glycid ether 100 (Epon 812), 10.000x magnification, kindly provided by H. Horstmann, Institute of Anatomy, University of Heidelberg

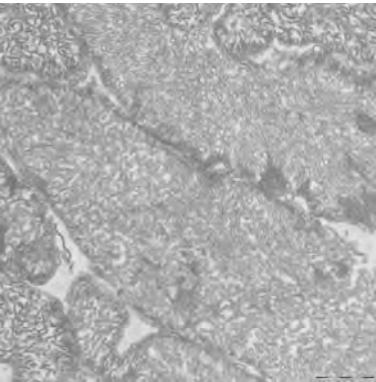


Figure 4: Retina from rat eye, embedding with Araldite CY 212, 26.000x magnification, kindly provided by H. Horstmann, Institute of Anatomy, University of Heidelberg

C. ARALDITE Embedding

Araldite® types are glycerol-based aromatic epoxy resins which show very little volume shrinkage after polymerisation. They show an essentially higher viscosity than ERL-4206, ERL-4221D and glycid ether 100. Compared with other epoxy resins, Araldite seems to be less grainy at very high resolution.

The former Araldite types CY 212, M and 502 are no longer produced. They are now substituted by Renlam M-1®, which has identical properties, but contains no toxic dibutyl phthalate.

Mixture according to Glauert (4)

Araldite CY 212 (Renlam M-1)	10 ml
Araldite HY 964 (DDSA) (hardener)	10 ml
Araldite DY 964 (DMP-30) (accelerator)	0.5 ml
Dibutyl phthalate	1.0 ml

Mixture according to Luft (9)

Araldite 502 (Renlam M-1)	27 ml
DDSA	23 ml
DMP-30	0.75 - 1 ml

Mixture according to Mollenhauer (10)

Araldite 502 (Renlam M-1)	15 ml
Glycid ether 100	25 ml
DDSA	55 ml
Dibutyl phthalate	2 - 4 ml
DMP-30 or BDMA	1.5 % - 3 %

Polymerisation can be done at 60 °C for 24 h.

Mixture for brain tissue embedding according to Horstmann (15)

Araldite CY212 (Renlam M-1)	7.5 ml
DDSA	27.5 ml
Glycid ether 100	15 ml
DMP-30 or BDMA	2 %

Mix well before adding DMP-30 or BDMA and immediately again after addition of the accelerator.

Mixture for retina embedding according to Horstmann (15)

Araldite CY 212 (Renlam M-1)	19 ml
DDSA	21 ml
Dibutyl phthalate	0.6 ml
BDMA	1.2 ml

Polymerisation time: 48 h at 65 °C

SERVA Reagents for Embedding

Description	Size	Cat. No.
ARALDITE® Hardener HY 964	100 g	13826.01
	1 kg	13826.02
BEEM capsules 5.2 mm for electron microscopy	100 pieces	43510.01
BEEM capsules 8.0 mm for electron microscopy	100 pieces	43511.01
Benzyl dimethylamine (BDMA)	10 ml	14835.01
D.E.R.® 736	100 ml	18247.01
Dimethylaminoethanol	5 ml	20130.04
	100 ml	20130.02
2-Dodecenylsuccinic acid anhydride (DDSA)	100 g	20755.01
	1 kg	20755.02
Embedding Medium ERL-4221 D	100 ml	21041.01
	250 ml	21041.02
	1 L	21041.03
Gelatin capsules No. 0 for electron microscopy	500 pieces	43520.02
Glycid ether 100 for electron microscopy	100 ml	21045.01
	500 ml	21045.02
Methylnadic anhydride (MNA)	100 ml	29452.01
	250 ml	29452.02
	1 L	29452.03
Modified SPURR Embedding Kit	1 Kit	21050.01
Nonenylsuccinic anhydride	250 ml	30812.01
	1 L	30812.02
PEG 4000	500 g	33136.01
	5 kg	33136.02
PEG 6000	500 g	33137.01
	5 kg	33137.02
Renlam® M-1	250 g	13825.01
	1 kg	13825.02
2,4,6-Tris(dimethylaminomethyl)phenol (DMP-30)	100 ml	36975.01
	250 ml	36975.03

ARALDITE® = registered trademark of Huntsman Advanced Materials Europe
Renlam® = registered trademark of Huntsman Advanced Materials Europe
D.E.R.® = registered trademark of Dow Chemical Company, USA.

5. Staining and Contrasting

Biological material is mainly composed of molecules containing atoms like carbon, hydrogen, oxygen, nitrogen, phosphor etc., which all have a low atomic weight and are therefore electron-transparent.

In the electron microscope, such untreated samples would yield a blurred image with low contrast. To

increase contrast, samples have to be treated with salts of heavy metals which are able to scatter electrons (5, 6, 8). There are two kinds of staining. In positive staining, the sample itself is stained. In negative staining, the surrounding medium is stained, mainly used to stain isolated macromolecules, viruses and cell organelles.

SERVA Products for Staining and Contrasting

Description	Size	Cat. No.
Ammonium molybdate	100 g	13370.01
3,3'-Diaminobenzidine • 4 HCl x H ₂ O (DAB)	100 mg	18865.01
	1 g	18865.02
Lead citrate x 3 H ₂ O	25 g	15158.01
Malachite green oxalate	25 g	28335.03
Osmium tetroxide for electron microscopy	100 mg	31251.01
	500 mg	31251.02
	1 g	31251.03
Osmium tetroxide 4 % solution for electron microscopy	2 ml	31253.01
	10 x 2 ml	31253.02
	10 ml	31253.03
	5 x 10 ml	31253.04
Phosphotungstic acid hydrate	25 g	32757.01
	100 g	32757.02
Silver nitrate	25 g	35110.01
	100 g	35110.02
Sodium molybdate x 2 H ₂ O	50 g	30207.01
Uranyl acetate x 2 H ₂ O	5 g	77870.02
	25 g	77870.01

6. Application Note: Specimen Preparation and Embedding of Rodent Brain Tissue ⁽¹⁵⁾

For the preparation of rodent brain tissue, it is at first necessary to fix the animals by perfusion. Cutting, contrasting and embedding can then be performed as described (3, 6).

1. Perfusion

Perfuse the left ventricle with 30 ml PBS, while the right auricle is opened by a cut of scissors. To obtain the right flow velocity, the solution has to be about 60 cm above the organ (corresponding to the blood pressure of rats in mm x Hg).

2. Fixation

Subsequently, 40 ml of the fixative (2.5 % glutaraldehyde/2 % paraformaldehyde in 0.1 M cacodylate buffer pH 7.4) are rinsed through with the same pressure. The brain tissue is then removed and kept overnight in the fixative solution at 4 °C. Tissue slices (about 200 µm thick) are cut on a vibratome and kept for 24 hours in PBS at 4 °C. Pieces of about 1.5 mm size are then excised with a razor-blade and washed 3 x 20 min in 50 mM cacodylate buffer pH 7.4. Postfix for 2 hours in 2 % osmium tetroxide/1.5 % potassium ferri-cyanide in water at room temperature and wash then for 2 hours in dist. water.

3. Blockstaining (8, 13)

Uranyl acetate dissolves more readily in methanol than in water or ethanol and methanolic solutions penetrate faster and deeper into the tissue than aqueous solutions. Therefore they are best suited for en bloc staining , resulting in a higher contrast.

The brain pieces are stained overnight at room temperature with 4 % uranyl acetate in 25 % aqueous methanol (bidest).

4. Dehydration:

Thereafter, the pieces are dehydrated in a methanol sequence followed by infiltration with the epoxy monomers:

2 x 30 min in 25 % methanol

2 x 30 min in 70 % methanol

2 x 30 min in 90 % methanol

2x 30 min in abs. methanol

5. Embedding:

Rotate the tissue for 3 hours in a mixture of Spurr resin and methanol 1:1. Leave over night at 4 °C in Spurr resin/methanol 3:1. Rotate for 3 hours at room temperature in pure Spurr resin. Embed in Spurr resin and polymerize for 48 hours at 70 °C.

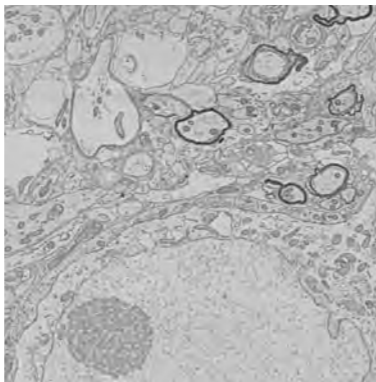


Figure 5: Calyx of Held from rat brain, embedding with Glycid ether 100 (Epon 812), 6.000x magnification, kindly provided by H. Horstmann, Institute of Anatomy, University of Heidelberg

SERVA Products Used in Specimen Preparation and Embedding of Rodent Brain Tissue

Description	Size	Cat. No.
Buffer Substance Dulbecco's (PBS)	10 L	47302.02
	50 L	47302.03
Cacodylic acid •Na-salt x 3 H ₂ O	25 g	15540.01
	100 g	15540.02
	500 g	15540.03
Glutaraldehyde 25 % solution in water for electron microscopy	25 ml	23114.01
	10x 5 ml	23114.02
Methanol for HPLC	2.5 L	45630.01
Modified SPURR Embedding Kit	1 Kit	21050.01
Osmium tetroxide 4 % solution for electron microscopy	2 ml	31253.01
	10x 2 ml	31253.02
	10 ml	31253.03
	5x 10 ml	31253.04
Paraformaldehyde	100 g	31628.01
	500 g	31628.02
Uranyl acetate x 2 H ₂ O	5 g	77870.02
	25 g	77870.01

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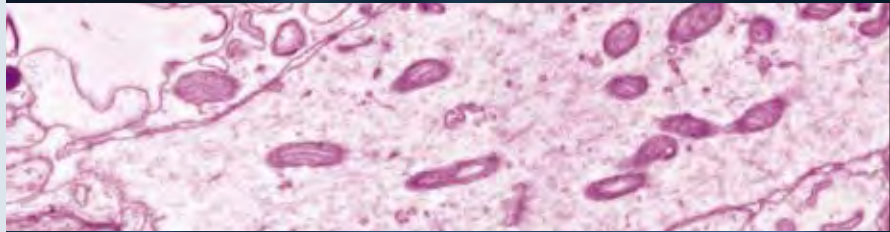
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