

Application Note

Leica EM AMW – Epoxy resin embedding of mouse tissues

Courtesy of:

Yannick Schwab
IGBMC-Centre d'Imagerie
Microscopie Electronique
1, rue Laurent Fries
67404 Illkirch Cedex

Living up to Life

Leica
MICROSYSTEMS

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Epoxy resin embedding of mouse tissues

Ultrastructural analysis

Epoxy resin embedding of mouse tissues (Y. Schwab, IGBMC, Illkirch, France)

i. Materials and reagents

Perfusion apparatus: peristaltic pump, tubing, blunted needle (18 gauge)

Syringes and needles

Dissection tools

Anesthesia : 1% ketamine, 0.2% xylazine in saline solution

Phosphate buffer :

Fixative : 2.5% glutaraldehyde and 4% formaldehyde in phosphate buffer

Osmium tetroxyde 1% in PB

Uranyl acetate 1% in d-water

Epoxy resin, for infiltration and embedding (45% epon812, 30% DDSA, 23% MNA, 2% DMP30)

Acetone

ii. Fixation protocol

Mice were anesthetized by intra-peritoneal injection of ketamine and xylazine and fixed by systemic perfusion of 15 ml fixative through the left ventricle (2 – 3 ml/min flow rate).

The different organs were dissected out and post fixed in the same fixative for 10 to 20 minutes. Pieces of 1mm³ were then cut with a sharp razor blade and processed for embedding in the automate.

iii. AMW programs

Reagent List

MaxTemp (°C)	MaxPower (watt)	Type	Name	DrainPause
50	30	Fixative	Buffer + glutaraldehyde	0
60	30	Rinse	Cacodylate buffer	0
50	30	Fixative	Buffer + OsO4	0
90	30	Rinse	dH2O	0
50	30	Fixative	Buffer + U-acetate	0
46	30	Solvent	Acetone 30%	0
46	30	Solvent	Acetone 50%	0
46	30	Solvent	Acetone 75%	0
46	30	Solvent	Acetone 95%	0
46	30	Solvent	Acetone abs.	0
50	30	Resin	Resin 3:1	30
50	30	Resin	Resin 1:1	10
50	30	Resin	Resin 1:3	20
95	30	Resin	Epon	50

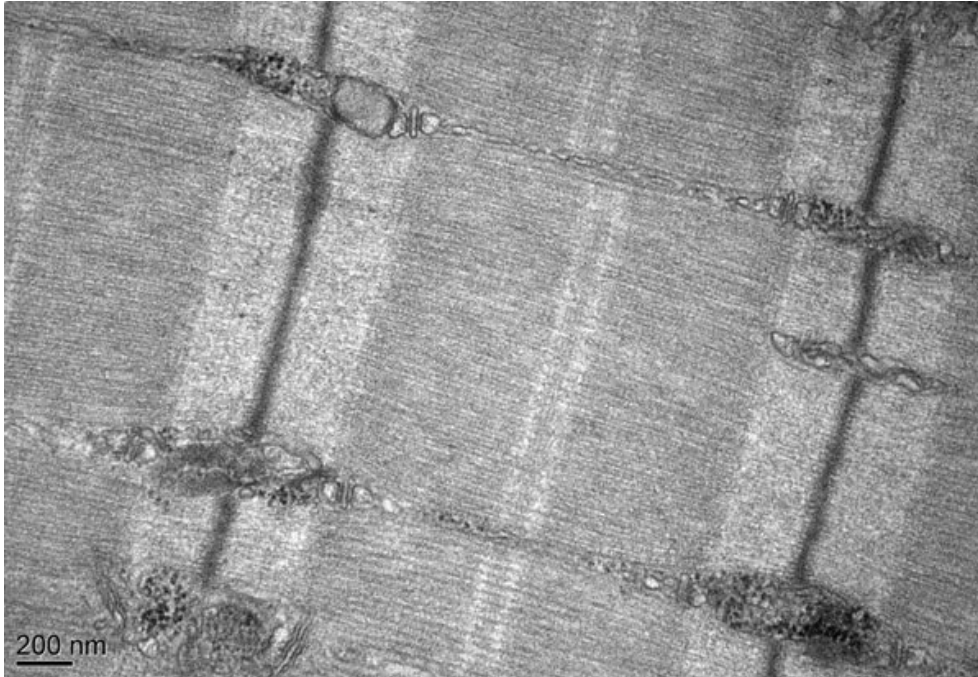
Epon embedding

Vial	Reagent	Power (watt)	Pause	Mode	Temp (°C)	Time (sec)
1	Buffer + glutaraldehyde	15		Continuous	37	120
1	Buffer + glutaraldehyde	0		Continuous	20	120
1	Buffer + glutaraldehyde	15		Continuous	37	120
1	Buffer + glutaraldehyde	0		Continuous	20	120
2	Cacodylate buffer	20		Slope	37	40
3	Cacodylate buffer	20		Slope	37	40
4	Buffer + OsO4	15		Pulse	37	720
5	dH2O	15		Continuous	37	60
6	dH2O	15		Continuous	37	60
7	Buffer + U-acetate	15		Continuous	37	120
	Buffer + U-acetate	0		Continuous	20	120
	Buffer + U-acetate	15		Continuous	37	120
8	dH2O	15		Continuous	37	60
9	Acetone 30%	20		Slope	37	60
10	Acetone 50%	20		Slope	37	60
11	Acetone 75%	20		Slope	37	60
12	Acetone 95%	20		Slope	37	60
13	Acetone abs.	20		Slope	37	120
14	Acetone abs.	20		Slope	37	120
15	Resin 3:1	10		Continuous	37	180
16	Resin 1:1	10		Continuous	40	180
17	Resin 1:3	10		Continuous	45	180
18	Epon	12		Continuous	50	180
19	Epon	12		Continuous	50	180
20	Epon	12		Continuous	50	180

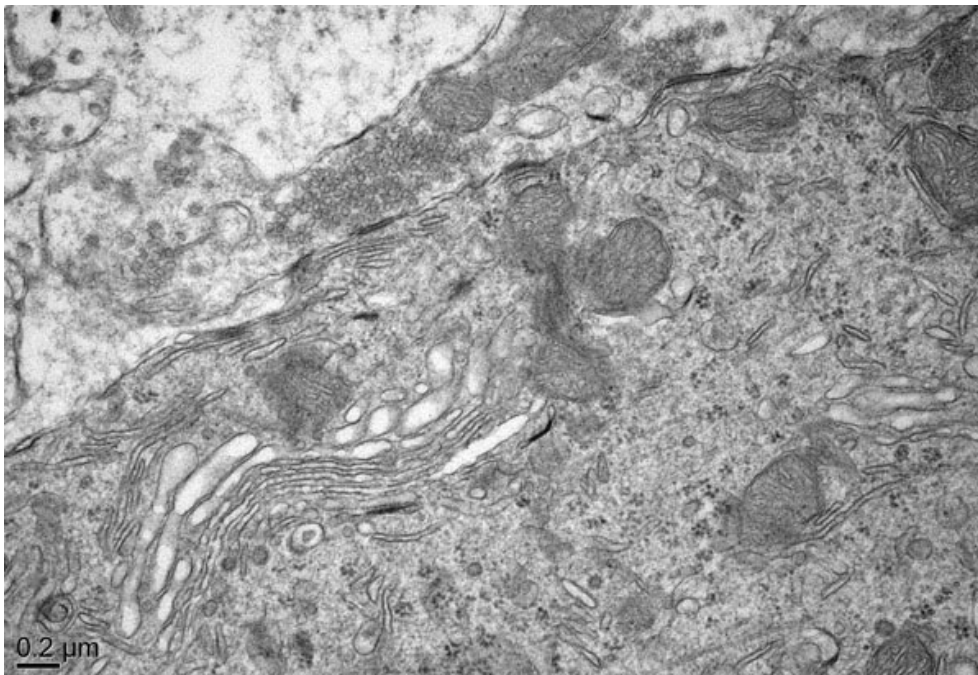
Poly Epon

Vial	Reagent	Power (watt)	Pause	Mode	Temp (°C)	Sec
1	Epon	30		Slope	63	300
1	Epon	30		Slope	75	300
1	Epon	30		Slope	83	900
1	Epon	30		Continuous	83	5700

iv. Pictures



Mouse skeletal muscle



Mouse cerebellum. *En passant* synapses on a Purkinje cell.