

## Protocol for the embedding of murine sciatic nerve for light microscopy using the LEICA EM Tissue Processor

I. Fischer and S. Reipert, Institute of Biochem. & Mol. Cell Biol., Vienna Biocenter, Dr. Bohr-Gasse 9, A-1030 Vienna

- Perfusion with 2.5% Paraformaldehyde + 0.5% Glutaraldehyde in PBS.
- Cutting the tissue in small pieces (1 mm<sup>3</sup>).

	<u>Reagent</u>	<u>Time</u>	<u>Temp.</u>	<u>Agitation</u>
1	3% glutaraldehyde in Sorensen's	over night	4°C	3
2	Sorensen's	10 min	R.T.	3
3	Sorensen's	10 min	R.T.	3
4	Sorensen's	10 min	R.T.	3
5	1% OsO <sub>4</sub>	90 min	R.T.	1
6	Sorensen's	10 min	R.T.	3
7	Sorensen's	10 min	R.T.	3
8	Sorensen's	10 min	R.T.	3
9	Ethanol 30 %	10 min	R.T.	3
10	Ethanol 30 %	10 min	R.T.	3
11	Ethanol 50 %	10 min	R.T.	3
12	Ethanol 50 %	10 min	R.T.	3
13	Ethanol 70 %	10 min	R.T.	3
14	Ethanol 70 %	10 min	R.T.	3
15	Ethanol 95 %	10 min	R.T.	3
16	Ethanol 95 %	10 min	R.T.	3
17	Ethanol 100 %	10 min	R.T.	3
18	Ethanol 100 %	10 min	R.T.	3
19	Propylene Oxide	10 min	R.T.	3
20	Propylene Oxide	10 min	R.T.	3
21	Agar 100: Propylene Oxide 1:2	15 min	R.T.	3
22	Agar 100: Propylene Oxide 2:1	30 min	R.T.	1

- Infiltration in pure epoxy resin (Agar100) over night.
- Fresh resin and polymerisation for 24 h at 60 °C.