

## Protocol for the embedding of mammalian tissues ( murine skeletal muscle) using the LEICA EM Tissue Processor

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- Perfusion with 2.5% Paraformaldehyde + 0.5% Glutaraldehyde in PBS.
- Cutting the tissue in small pieces (1 mm<sup>3</sup>).
- Fixation in 3% Glutaraldehyde in Sorensen´s over night at 4°C (tissue already placed in TP baskets).

	<u>Reagent</u>	<u>Time</u>	<u>Temp.</u>	<u>Agitation</u>
1	1.5 % OsO <sub>4</sub>	90 min	R.T.	3
2	Sorensen´s	5 min	R.T.	3
3	Sorensen´s	5 min	R.T.	3
4	Sorensen´s	5 min	R.T.	3
5	Sorensen´s	5 min	R.T.	3
6	Ethanol 30 %	5 min	R.T.	3
7	Ethanol 30 %	5 min	R.T.	3
8	Ethanol 50 %	5 min	R.T.	3
9	Ethanol 50 %	10 min	R.T.	3
10	Ethanol 70 %	5 min	R.T.	3
11	Ethanol 70 %	10 min	R.T.	3
12	Ethanol 95 %	5 min	R.T.	3
13	Ethanol 95 %	10 min	R.T.	3
14	Ethanol 100 %	4 min	R.T.	3
15	Ethanol 100 %	4 min	R.T.	3
16	Ethanol 100 %	4 min	R.T.	3
17	Ethanol 100 %	4 min	R.T.	3
18	Propylene Oxide	5 min	R.T.	3
19	Propylene Oxide	5 min	R.T.	3
20	Propylene Oxide	5 min	R.T.	3
21	Propylene Oxide	15 min	R.T.	3
22	Agar 100: Propylene Oxide 1:2	1 hr	R.T.	3
23	Agar 100: Propylene Oxide 2:1	2 hrs	R.T.	3
24	Pure Agar 100	o/n	R.T.	0

- Fresh resin and polymerisation for 24 h at 60 °C.