Dehydration and paraffin embedding of tissues for histology

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The purpose of embedding biological specimens is to replace water by a matrix which is sufficiently stable to maintain cell structures. For this purpose, tissue blocks are first stabilized by fixatives (a selection of routine fixations is given under "*Fixation methods*". Then, specimens are dehydrated and embedded in paraffin wax which is the typical embedding medium for routine histology. A modern paraffin embedding medium for tissue specimens is Paraplast® or Paraplast® Plus (registered trademark of Sherwood Medical to be purchased from many commercial sources). Paraplast is a highly purified paraffin with a melting point of 56ºC and formulated to give outstanding tissue infiltration and superior quality sections. Paraplast Plus is similar to Paraplast with a slight variation in composition: it is composed of paraffin wax (>98%), polyisobutylene (<1%) and dimethylsulfoxide (<1%). Unlike some other tissue embedding products, Paraplast is already doubly filtered, and there is no need for further filtration prior to use. Such paraffin embeddings have proven very useful for the preparation of tissue sections by microtomes and subsequent application of many staining techniques including immuno-staining and other molecular specific labellings. Typical schedules are given in the following chapters. *

Chemicals *p.a*. are used according to the recommendations of the manufacturer:

Ethanol, 100 %

Chloroform

Xylene or xylene substitute such as Histoclear®

Paraplast®

Paraffin embedment, routine method after formaldehyde fixation (tissue blocks at room temperature)

Procedure	Reagent	Time
1	70 % v/v ethanol in distilled water	1 h
2	70 % v/v ethanol in distilled water	1 h
3	80 % v/v ethanol in distilled water	1 h
4	96 % v/v ethanol in distilled water	1 h
5	96 % v/v ethanol in distilled water	1 h
6	100 % ethanol	1 h
7	100 % ethanol	1 h
8	100 % ethanol	1 h
9	100 % ethanol	1 h
10	chloroform or xylene or xylene substitute	1 h
11	chloroform or xylene or xylene substitute	1 h
12	chloroform or xylene or xylene substitute	1 h

* Chemicals for dehydration and embedment can be toxic. They must be handled with care

13	paraffin wax (e.g. Paraplast)	2 h at 56-58°C
14	paraffin wax (e.g. Paraplast)	2 h at 56-58°C
15 (vacuum)	paraffin wax (e.g. Paraplast)	2 h at 56-58°C
16	embedment: tissue blocks are transferred into appropriate molds at 58°C; block-out when cooled down	-

Paraffin embedment, method after ethanol-acetic acid fixation(96-99% ethanol-1% acetic acid at 0-4°C, tissue slices ca. 0.5 cm thick)

Procedure	**Reagent**	**Time**
1	100 % ethanol	20 min at 0-4°C
2	100 % ethanol	20 min at 0-4°C
3	100 % ethanol	60 min at room temperature
4	100 % ethanol	60 min at room temperature
5	100 % ethanol	60 min at room temperature
6	chloroform or xylene or xylene substitute	60 min at room temperature
7	chloroform or xylene or xylene substitute	60 min at room temperature
8	chloroform or xylene or xylene substitute	60 min at room temperature
9	paraffin wax (e.g. Paraplast)	90 min at 56-58°C
10	paraffin wax (e.g. Paraplast)	90 min at 56-58°C
11 (vacuum)	paraffin wax (e.g. Paraplast)	120 min at 56-58°C
12	embedment: tissue blocks are transferred into appropriate molds at 58°C; block-out when cooled down	-

References for further readings

Romeis B (1968)
Bancroft JD *et al*. (1994)
Bancroft JD and Gamble M (2007)

Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

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