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HuBMAP - Tissue Processing with Paraffin Infiltration

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for the automated processing of formalin fixed HuBMAP specimens for paraffin embedding.

GUIDELINES

- Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
- Laboratory personnel - are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

MATERIALS

NAME	CATALOG #	VENDOR
Paraplast	39501006	
100% EtOH	DSP-CA-151	Gold Shield Chemicals
Xylene	X3P-1GAL	Fisher Scientific
Sakura® VIP 6 Tissue Processor	VIP 6	Sakura Finetek
Tissue Cassette Basket	# 5112D	Newcomer Supply

MATERIALS TEXT

10% Neutral Buffered Formalin (NBF)
 Paraffin Wax (Paraplast X-tra)
 Xylene
 Ethanol (EtOH)- 70%, 95%, 100%
 Tissue Processor (automatic)
 Tissue Processing Cassette Basket

SAFETY WARNINGS

- As these specimens are both human in origin and immersed in formaldehyde, gloves and other PPE are required.
- Chemicals used during processing are flammable (ethanol and xylenes). Do not overfill reagent bottles.
- Paraffin melting point cannot exceed 70C.
- Oven and paraffin containers are hot, use extreme care.
- Never open retort lid if vacuum or pressure is being displayed- open only when ambient is displayed.

BEFORE STARTING

- Confirm processor is on, and reagent bottles are filled to specified volumes (indicated on labels).
- Check that reagent bottle screw caps are tightened

- 1 Ensure that the retort is drained and the Clean Cycle has been run. If not, perform Clean Cycle.

Processing Selection & Initial Steps

- 2 Place dividers in processor sample basket and load cassettes. No more than 10 cassettes can be added to each partition. Cover^{1d} the basket with the basket lid.



Perform transfer of cassettes in the hood to avoid inhalation of the 70% ethanol that the cassettes are held in prior to procesing

- 3 Open the retort and place the basket inside. Rotate handles until they lay flat against the sides of the chamber. Close and latch^{1d} retort

- 4 Press "Tissue Processing" on Main Menu. Enter desired program number (program used listed below). Select Immediate Start or Delayed Start to control the beginning of processing so that completion coincides with availability to embed at termination.^{3d}



Appendix 1

Paraffin Fixation and Processing Program

Station #	Solution*	Conc.	Time	Set Temp.	P/Vacuum	Mixer
1	EtOH	70%	Hold**	_____	off	off
2	EtOH	70%	0:35	_____	P/V	slow
3	EtOH	80%	0:35	_____	P/V	slow
4	EtOH	95%	0:35	_____	P/V	slow
5	EtOH	95%	0:35	_____	P/V	slow
6	EtOH	100%	0:35	_____	P/V	slow
7	EtOH	100%	0:35	_____	P/V	slow
8	Xylene	100%	0:45	_____	P/V	slow
9	Xylene	100%	0:45	_____	P/V	slow
10	Paraffin	_____	0:45	60°C	P/V	slow
11	Paraffin	_____	0:45	60°C	P/V	slow
12	Paraffin	_____	0:45	60°C	P/V	slow
13	Paraffin	_____	0:45	60°C	P/V	slow

*Maintenance if performed according to manufacturer's recommendation.

**Duration of holding time dependent on desired end time to start embedding.

- 5 When processing is completed remove basket and press "Drain Retort"
- 6 Transfer cassettes from the processing basket to the embedding station paraffin chamber.



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