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## HuBMAP - Tissue Processing with Paraffin Infiltration

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**We use this protocol and it's working**

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## 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

### MATERIALS

✕ Paraplast **Catalog #39501006**

✕ 100% EtOH **Gold Shield Distributors Catalog #DSP-CA-151**

✕ Xylene **Fisher Scientific Catalog #X3P-1GAL**

✕ Sakura® VIP 6 Tissue Processor **Sakura Finetek Catalog #VIP 6**

✕ Tissue Cassette Basket **Newcomer Supply Catalog ## 5112D**

10% Neutral Buffered Formalin (NBF)

Paraffin Wax (Paraplast X-tra)

Xylene

Ethanol (EtOH)- 70%, 95%, 100%

Tissue Processor (automatic)

Tissue Processing Cassette Basket



## Troubleshooting

## Safety warnings



- As these specimens are both human in origin and emmersed 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 a,mbient is displayed.

## Before start

- Confirm processor is on, and reagent bottles are filled to specified volumes (indivxcated on labels).
- Check that regent 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 the basket with the basket lid.

1d

### Note

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

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

1d

- 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

Note

**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	<div><div></div><div></div></div>	0:45	60°C	P/V	slow
13	Paraffin	<div><div></div><div></div></div>	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.