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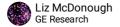
♠ HuBMAP | Formalin Fixation and Paraffin Embedding of Tissue Samples

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2 Works for me dx.doi.org/10.17504/protocols.io.bqp6mvre

Human BioMolecular Atlas Program (HuBMAP) Method Development Community | GE Research



ABSTRACT

This method details formalin fixation and paraffin embedding of the HuBMAP tissue specimens.

Protocol adopted from *Marda Jorgensen, Jerelyn Nick (02/24/2020). HuBMAP: Paraffin Embedding Tissue Samples . https://dx.doi.org/10.17504/protocols.io.bam9ic96*

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MATERIALS TEXT

Instrumentation

Α	В
Tissue Processor Leica Peloris II	
Embedding Center Leica Embedding Center with Cryo-console (Model EG	

Materials/Reagents

Α	В		
Materials	Catalog Number/Model		
Formalin	Fisher Scientific		
Ethyl Alcohol	State of Pennsylvania Alcohol Control Board		
Xylene	Fisher Scientific		
Embedding media/paraffin	Surgipath Formula R (3801470)		
4x4 Gauze	Fisher Scientific (1400249)		
Embedding molds	Leica Stainless Steel embedding molds (39LC-700-2)		
Curved forceps	Fisher Scientific (16-100-110)		

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BEFORE STARTING

- It is essential to read the complete instruction booklet before starting work.
- Users must be properly trained.
- Equipment and facility must be maintained and in good working order.

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- During fixation, formalin will penetrate the tissue causing chemical and physical changes that harden and preserve the tissue's morphology and protect it against subsequent processing steps.
- Because melted paraffin wax is hydrophobic, most of the water in a specimen must be removed before it can be
 infiltrated with wax, so the tissue must go through dehydration in a series of ethanol solutions.
- Xylene is then used to clear the tissue, displacing the ethanol which will be in turn displaced by molten paraffin wax.

TISSUE PROCESSING

https://dx.doi.org/10.17504/protocols.io.bqp6mvre

7 Tissue processing is done using the Leica Peloris II using the following program.

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Α	В	С
Formalin	30 minutes	Ambient temperature
75% Ethanol	30 minutes	37 degrees C
80% Ethanol	30 minutes	37 degrees C
95% Ethanol	30 minutes	37 degrees C
95% Ethanol	30 minutes	37 degrees C
100% Ethanol	45 minutes	37 degrees C
100% Ethanol	50 minutes	37 degrees C
100% Ethanol	60 minutes	37 degrees C
Xylene	30 minutes	37 degrees C
Xylene	30 minutes	37 degrees C
Xylene	40 minutes	37 degrees C
Xylene	60 minutes	37 degrees C
Paraffin	30 minutes	60 degrees C
Paraffin	60 minutes	60 degrees C
Paraffin	90 minutes	60 degrees C

Tissue processing program the Leica Peloris II

POST-PROCESSING

2	Processed cassette should be	placed in wax holding chambe	er of embedding center.
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4	Pre-heat embedding utensils in the forceps warming station; warming chambers are located on the side of the paraffin
	dispensing nozzle.

5	Turn	on	tho.	ototion	مماط	nlata	module.
5	Turn	OH	me	station	cold	piate	module.

6	Open the cassette.	Be sure to carefully maintain t	the tissue orientation. Dispose of cassette lid	l.

- 7 Select an appropriate sized embedding mold.
- 8 Partially fill a mold with molten paraffin by using forceps to tap the dispenser plate on the embedding center's dispensing nozzle.
- 9 Carefully transfer tissue into the center of the mold using heated forceps. The tissue should be placed cut side down.
- 10 Transfer the mold to the cold pad and gently press tissue flat with forceps, allowing the paraffin to solidify in a thin layer.

11	Place the lower basket of the emptied cassette on top of the mold and add additional molten paraffin to fill 2/3 of the
	cassette basket. If there are bubbles, slowly lift and reposition the cassette to remove.

12	Transfer the paraffir	filled mold to the cold	plate module and allow to solidify
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