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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

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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**

| A | B |
|---|---|
| Tissue Processor | Leica Peloris II |
| Embedding Center | Leica Embedding Center with Cryo-console (Model EG1160) |

Materials/Reagents

| A | B |
|---|---|
| **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

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



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| A | B | C |
|---|---|---|
| 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

3 Processed cassette should be placed in wax holding chamber of embedding center.

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 the station cold plate module.

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

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.



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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 paraffin filled mold to the cold plate module and allow to solidify.



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