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FFPE Blocking and Sectioning - UMN TMCs

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Colleen Forster, HT(ASCP)QIHC¹, David A Bernlohr¹, Laura J Niedernhofer¹

¹University of Minnesota, Minneapolis, MN

Cellular Senescence Net...



Allie Abdellatif

MIBAM, University of Minnesota, Minneapolis, MN

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Protocol status: Working

We use this protocol and it's working

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Abstract

FFPE Blocking and Sectioning protocol from BLS Histology at the University of Minnesota.



BLS Tissueprocessing protocl 1 ove... 50KB



FFPE Blocking

1 Specimen Preparation:

The tissues for processing should be well fixed, rinsed in running tap and placed into 80% alcohol. The suggested fixation is perfusion with 4% paraformaldehyde, fresh tissue dropped into 4% paraformaldehyde or 10% formalin for 24-48 hours or alcohol fixed tissues. To improve fixation, fix small pieces of tissue so solution can penetrate better.

2 Label cassettes using a pencil.

3 Block tissues according to researcher requests.

4 Place into labeled cassette, cover and place into container under running tap water.

5 Rinse well.

6 Drain tap water and replace with 80% alcohol

7 Bring to Rm2-155 Jackson Hall for processing.



<u>Station</u>	<u>Solution</u>	<u>Time</u>
#1 ON		
1	80% ethanol	40 min
2	95% ethanol	40 min
3	95% ethanol	40 min
4	100% ethanol	40 min
5	100% ethanol	40 min
6	100% ethanol	40 min
7	100/xylene 50/50	30min
8	xylene	60min
9	xylene	90min
10	xylene	30min
11	paraffin	30min
12	paraffin	60min
13	paraffin	90min
14	paraffin	30min
		11hr

- 8 Place cassettes in paraffin bath at embedding center
- 9 Cover with paraffin.
- 10 Move to cold plate for hardening.
- 11 Pop cooled block out of mold and trim edges.
- 12 Block is ready to cut.
- 13 When cutting the tissue, prepare sections according to the Histology Request sheet.

FFPE Slide Sectioning

- 14 Remove excess wax from the sides of the plastic cassettes by scraping or melting.



- 15 Orient the block in chuck holder
- 16 Begin carefully cutting into paraffin block until a full face of tissue is exposed
- 17 Place block onto ice/water and soak for 15 minutes or longer if necessary
- 18 Cut section at desired thickness ensuring proper orientation
- 19 Carefully pick up section and place in a water bath
- 20 Place tissue section on required glass slide
- 21 Air dry for minimum of 1 hour and then move to the 37 degree oven
- 22 Dry slides overnight.