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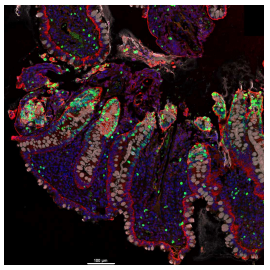
# Immuno Fluorescence staining of human FFPE (formalin fixed paraffin embedded) gut mucosal biopsy



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Human Cell Atlas Metho... | Helmsley project_Basu lab

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**Protocol status:** Working
**I use this protocol and it's working**

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## Disclaimer

This protocol is for research only.

## Abstract

This protocol is to provide a detail instruction on the immuno fluorescence staining on human gut mucosal biopsy that has been preserved in FFPE and sectioned at 5 micron thickness. The fixation for the tissues was 24 hour at room temperature in 10% neutral buffered formalin. Target retrieval needs optimization is fixation, sectioning conditions change.

## Guidelines

The human gut mucosal tissues was retrieved by colonoscopy. The size of tissues range from 1 × 1 × 1 mm, to 2 × 4 × 4 mm.
Smaller tissues were embedded in 1% agarose gel before FFPE.

## Materials

1x Citrate buffer 200 ml
20 ml 10x Citrate buffer
180 ml distilled water

1x PBST 1000 ml
100 ml 10x PBS
0.5ml 10% Tween20
900ml distilled water

Permeabilization buffer 5 ml
125ul 10% TritonX-100
3.3 1x PBST
0.5ml 10% BSA

Blocking buffer 5 ml
2.5ml 10% BSA
2.5ml 1x PBST

Histoclear II (National diagnostics HS-202)
Histology grade ethanol (VWR 89370-084)
10xCitrate buffer (MillieporeSigma C9999)
10xPBS (FisherSci BP399500)
10%Tween20 (Teknova T0710)
10%TritonX (MillieporeSigma 93443)
10%BSA (Miltenyi Biotec 130-091-376)

## Safety warnings

Boiling buffer, microwave and hotplate are used in the protocol. Please proceed with caution and use thermal gloves at these steps.

## Ethics statement

The human intestinal tissue are obtained after patients' consents and approval from Institutional Review Board at the University of Chicago (IRB Number: 15573A). All the samples are processed for research use only.

## Before start

Check the tissue block quality with H&E histology.

## Deparaffinization

15m

1 Immerse tissue sections on slides in Histoclear II in a staining dish Room temperature 00:05:00 . Move the slide holder up and down x 5 times. Repeat this step two more times with fresh Histoclear II. There should be no visible paraffine remaining on the slide by the end of this step. 15m

## Rehydration

16m

2 Move to another staining dish. Shake off Histoclear II from former step. Immerse tissue sections on slides in histology ethanol in a staining dish Room temperature 00:02:00 . Repeat this step one more times with fresh ethanol. 4m

3 Move to another staining dish. Shake off ethanol from former step. Immerse tissue sections on slides in 70% histology ethanol in a staining dish Room temperature 00:02:00 .Repeat this step one more times with fresh 70% ethanol. 4m

3.1 Prepare fresh 1 x Citra buffer 200 ml in a glass beaker, and place 200 ml distilled water in the second glass beaker.

4 Move to another staining dish. Shake off ethanol from former step. Immerse tissue sections on slides in 50% ethanol in a staining dish Room temperature 00:02:00 . Repeat this step one more times with fresh 50% ethanol. 4m

4.1 Microwave the liquid in the beakers x 1 minute. Place the beakers with liquid on the hot plate and bring the liquid to the boiling point.

5 Move to another staining dish. Immerse tissue sections on slides in distilled water in a staining dish Room temperature 00:02:00 . Repeat this step one more times with fresh water. 4m

## Target retrieval

26m 30s

6 Place the slides to a slide basket. Immerse the tissue sections in the boiling water 100 °C . 30s

7 Move the slides from boiling water. Immerse the tissue sections in boiling 1 x citrate buffer 100 °C . There should be no agarose gel remaining on the slides. 15m

8 Move the slides from citrate buffer. Cool the tissues in distilled water dish Room temperature . 1m

8.1 Remove all the beakers from the hot plate.

9 Airdry the slides Room temperature . 5m

10 Draw a hydrophobic barrier around the tissue sections on the slides. 5m

10.1 Prepare the humidity chamber with the heated water from step 13.

## Permeabilization 22m

11 Place the slides in a humidity chamber. Add 1 x PBS to the tissues Room temperature . 2m

12 Tap off water. Add permeabilization buffer to the tissues Room temperature 00:10:00 . Repeat one more time with fresh permeabilization buffer. 20m

## Blocking and primary antibody incubation 17h 30m

13 Tap off the buffer. Add blocking buffer to the tissues Room temperature . 1h

13.1 Prepare the primary antibody dilutes in 1 x PBST.

14 Tap off the blocking buffer. Add antibody dilutes to the tissues 4 °C Overnight . 16h

15 Tap off buffers. Immerse the slides in a staining dish with 1 x PBST Room temperature 00:10:00 on a horizontal shaker. Repeat the rinse for two more times. 30m

15.1 Prepare the secondary antibody dilutes in 1 x PBST.

## Secondary antibody incubation and mounting 1h 15m

16 Tap off PBST. Place the slides in the humidity chamber. Add antibody dilutes to the tissues Room temperature 01:00:00 . 1h

17 Tap off buffers. Immerse the slides in a staining dish with 1 x PBST Room temperature 00:05:00 on a horizontal shaker. Repeat the rinse for two more times. 15m

17.1 If nuclei staining is need, incubate tissues with DAPI for 2 minutes at room temperature between the second and third rinses.

18 Tap off buffer. Add prolong Gold to the tissues and cover with cover glass.