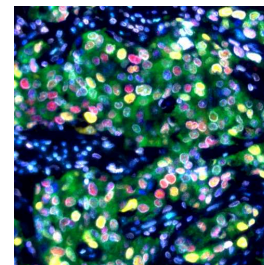


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## mxIF protocol

DOI

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**Protocol status:** Working

We use this protocol and it's working



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## Abstract

This protocol is developed by SenNet JHU TMC group

## Troubleshooting



## Dewaxing and rehydration

2h 5m

- 1 Tissue slides are baking 1 hour before dewaxing 1h
- 2 Tissue dewaxing is performed with 5 minutes sequential wash in 3 times 100% xylene, 3 times 100% EtOH, followed by 90%, 80%, and 75% EtOH 1h
- 3 Tissue slides are then dipped in water several times to finish rehydration 5m

## Heat antigen retrieval

1h

- 4 Heat antigen retrieval steps are performed with 300 ul of unmasking solution in 32 ml of H<sub>2</sub>O in a steamer for 20 minutes 40m

## Blocking

30m

- 5 Blocking is performed with Peroxidase and Alkaline Phosphatase Blocking reagent for 15 minutes 30m

## Primary antibody incubation

- 6 Primary antibody is incubated 1 hr at room temperature or 4 C overnight, depending on targeted protein

## Signal amplification

- 7 Slides are incubated with 1 drop of anti-ms HQ (or anti-rb HQ depends on primary antibody host animal) at room temperature for optimized time
- 8 Slides are incubated with 1 drop of anti-HQ HRP at room temperature for optimized time
- 9 TSA dyes are incubated for optimized time



## Antibody removal

- 10 Antibody removal kit or heat mediated antibody stripping is applied to remove the primary antibody

## Multiplex IF

- 11 Repeat step 5-10 until all the fluorescent channels are occupied
- 12 Counter stain the slides and imaging with fluorescent microscope

## Bleaching

- 13 Bleaching is performed with 4.5% of H<sub>2</sub>O<sub>2</sub> and 24mM NaOH made up with PBS (CyclIF bleaching protocol)
- 14 Repeat step 5-13 for different rounds of imaging