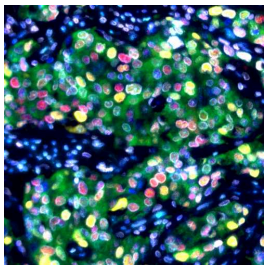
Mar 20, 2024

mxIF protocol



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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 H2O 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 H2O2 and 24mM NaOH made up with PBS (CycIF bleaching protocol)

14 Repeat step 5-13 for different rounds of imaging