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**Immunofluorescent staining from paraffin samples**

If you are going to do ISH (RNA detection) together with IHC, all materials should be prepared RNAase free.

Materials

Histo-clear (https://www.nationaldiagnostics.com/histology/product/histo-clear)

Ethanol

Antigen unmasking solution (https://vectorlabs.com/antigen-unmasking-solution-high-ph.html)

1. deparaffinized

①put samples into Histo-clear for 10min

②put samples into100% ethanol for 5min (twice volume of Histo-clear to remove it)

③put samples into 95% ethanol for 1min (Critical to keep the tissue to be GOOD)

④put samples into 85%（or 75%） ethanol for 1min

⑤put samples into 50% ethanol for 1min

⑥ Wash slides with dI water x3 to remove EtOH/Histo Clear as much as possible.

2. antigen retrieval

①put the sample into the cambers filled with antigen unmasking solution 1.88ml/200ml (high pH: from Victor Cat#: H-3301 )

Alternatively, you can make cheaper buffer by yourself:

1M Tris-HCL pH 8.8 2.92 ml + 0.5M EDTA 0.4ml in 200ml DI water

②Heat the sample in a pressure cooker at 110 degree for 15 min

③Take samples out from the pressure cooker around 1-1.5h after starting (Appropriate temperature to remove out the sample is around 80~90degree)

④put samples into PBS

3. blocking (Option)

①Mark around the samples by Puppen, put the blocking buffer (Rodent blocker)for 30min

②wash the samples using PBS

4.1st antibody

①put the antibody (1:100) onto the samples

②incubate them overnight at 4℃

5.2nd antibody

①wash the samples using PBS

②put the 2ndary antibody (1:300) onto the samples

③incubate them for 1.5h~2hrs. at room temp.