Immunofluorescence staining protocol

Material:

- 3 µm FFPE-cuts on Advanced Adhesive StarFrost® slides
- Xylol
- Decreasing ethanol series: 100 %, 100 %, 96 %, 80 %, 70 %, 40 %, ddH_2O (deionized water)
- PBS (preparation see appendix, store at room temperature)
- Plastic box (e.g. from Ikea) + lid with holes for antigen retrieval
- Citrate buffer (pH 6, mix of solution A & B, for preparation see appendix)
    - solution A: 14.4 ml
    - solution B: 65.6 ml
    - ddH_2O: 720 ml
- PAP Pen
- Ice
- Humid chamber
- 10x Rotiblock (Carl Roth, A151)
- TritonX (e.g. SERVA Electrophoresis GmbH, 37240)
- Blocking/permeabilization buffer: 1x Rotiblock in ddH_2O / 0.1% TritonX (fill into drop bottle, store at 4 °C)
- Staining buffer: 1x Rotiblock in ddH_2O (store at 4 °C)
- 1^{st} antibody: antibody-specific concentration
- 2^{nd} antibody: antibody-specific concentration (for AlexaFluor fluorophor from Invitrogen 1:500)
- DAPI (Thermo Fisher Scientific, D3571)
- Fluorescence Mounting Medium (Agilent Technologies, S302380)
- Cover slips

Method:

I) Deparafination & dehydration

- Xylol 3 x 5-8 min
- Decreasing ethanol series: 100 %, 100 %, 96 %, 80 %, 70 %, 40 %, 1 min each
- Wash in ddH_2O

II) Antigen unmasking:

- Put slide holder into plastic box with citrate buffer (always two holders à 10 glass slides per plastic box)
- Microwave (microwave-specific settings, boiling time should be ~20 min)
- Cool whole box on ice for at least 15 min (possible pause point)
- Wash slides 1 x 5 min in PBS

→ Encircle cuts with PAP Pen; apply blocking buffer drops immediately (do not let cuts dry out!)

Incubation with **80 - 100 µl/ cut** in humid chamber
All steps at room temperature

III) Staining

step	reagent	time
Blocking	Blocking/permeabilization buffer	1 h
1st Antibody	Antibody-specific concentration in staining buffer	1 h at room temperature OR over night at 4 °C
Wash	PBS	2 x 5 min
2nd Antibody	In staining buffer	1 h
Wash	PBS	3 x 5 min
Counter-staining	DAPI (1:1000 in PBS)	10 min
Wash	PBS	3 x 5 min
Coverslip	Fluorescence mounting medium	
Dry	In the dark with open lid!	Over night

Afterwards store at 4 °C for up to 1 month or, for several months, at -20 °C (tape seal box air-tight)

Appendix:

- **PBS:**

NaCl	36 g
$Na_2HPO_4 + 2\ H_2O$	9.4 g
KH_2PO_4	2.15 g

  add 5 l ddH_2O, shake and let settle for at least 1 h
  shake again before first use

- **Citrate buffer** (prepare solutions in advance and store at 4 °C)
  solution A: 14.4 ml

Citric acid	21.01 g
ddH_2O	1000 ml

  solution B: 65.5 ml

Sodium citrate	29, 41g
ddH_2O	1000 ml

  ddH_2O: 720 ml