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Immunofluorescence of cryopreserved tissue sections

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

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Abstract

Protocol for immunostaining cryopreserved tissue sections

Materials

	A	B	C
	Reagent ID	**Vendor**	**Catalog #**
	Bovine Serum Albumin (BSA)	Sigma	A3059-50G
	Saponin	Sigma	47036-50G-F
	AffiniPure Fab Fragment Anti Mouse IgG (H+L) (Fc Block)	Jackson ImmunoResearch	115-007-003
	TrueBlack	Biotium	23014
	ProLong Diamond anti fade mountant	Invitrogen	P36961
	Fluormount-G	Invitrogen	00-4958-02
	Hoechst 33342	Invitrogen	H3570
	Triton X-100	Sigma	T8787-100ML

PROTOCOL FOR TISSUE IFA - CRYOPRESERVED TISSUE SECTIONS

2h 51m

1 2h 51m

1. Wash slides 2x with PBS (00:03:00) to remove OCT.

(Optional) Delimitate a circular area around the tissue slice using a hydrophobic slide marker. All the following incubation steps can be done in 50-100 µL total volume (enough for covering the tissue slice). If using more than one tissue slice per slide and different primary antibody mixes make sure the mixes are well separated.

2. Block/permeabilize with PBS/ 1% BSA/ 0.1% Saponin (or 0.2% Triton X-100 diluted in PBS/1% BSA) + **Fc Block (1:200)** for 00:40:00 at room temperature.

a) No need for permeabilization if staining just for surface proteins. Remove the permeabilization reagent (Saponin or Triton) from the block mixture accordingly.

b) No need to wash before moving to the next step. Gently tap the slide against the paper towel to remove the blocking solution.

3. Incubate with **primary antibody mixes** for 01:00:00 at Room temperature - mixes prepared in PBS/ 1% BSA/ 0.1% Saponin (or in PBS/ 1% BSA if not staining intracellular proteins).

a) Incubation with primary antibodies can be done at 4 °C Overnight to improve staining.

4. Wash 3x with PBS (00:03:00)

5. Incubate with **secondary antibodies** - prepared in PBS/ 1% BSA/ 0.1% Saponin (or in PBS/ 1% BSA if not staining intracellular proteins) - for 00:40:00 at Room temperature

6. Wash 3x with PBS (00:03:00)

7. Nuclear staining - Incubate with **Hoechst** (diluted 1 µL in 10 mL of PBS) for 00:10:00 at Room temperature

8. Wash 3x with PBS (00:03:00)

9. Background quenching - Incubate with 1x TrueBlack (diluted to 1X in 70% EtOH) for 00:03:00 at Room temperature to reduce tissue autofluorescence.

10. Wash 2x with PBS (00:03:00) and once with dH2O (00:03:00)

11. Mount with coverslips and Prolong Antifade mounting fluid or Fluormount-G.

Protocol references

Annotated by IAC - Updated by Lavinia Gonzalez