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IMMUNOFLUORESCENCE PROTOCOL FOR FROZEN TISSUE

This protocol is a recommendation only. Please optimize the procedure since experimental conditions can vary for different tissue samples.

TISSUE PREPARATION

Note: For tissue stored at -80°C, remove from freezer and equilibrate at -20°C for about 15 minutes before sectioning. This prevents cracking of the block during sectioning.

1. Cut sections at 4-8 um and place on pre-cleaned and positively charged microscope slides.
2. Air dry sections on bench for a few minutes to help the sections adhere to the slides.
3. Fix sections in precooled acetone for 10 minutes at 4°C.
4. Wash with Tris-Buffered Saline (TBS) to remove all traces of acetone.

IMMUNOSTAINING

Recommended: Do not allow tissues to dry at any time during the staining procedure.

1. Rinse slides 2 times with Tris-Buffered Saline + Tween (TBST) for 5 minutes each at RT.
2. Block with 5% serum or BSA for 2 hours at RT.
3. Drain blocking buffer from slide.
4. Incubate slides with the diluted primary antibody overnight at 4°C with gentle agitation.
5. Wash slides 2 times with TBST for 5 minutes at RT.

   Note: If using a primary conjugated antibody skip to step 12.

1. Incubate slides with diluted conjugated secondary antibody for 2 hour at RT with gentle agitation.
2. Wash slides 2 times with TBST for 5 minutes at RT.
3. Mount coverslips.