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Free Floating IHC Fluorescent Staining

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Protocol status: Working

We use this protocol and it's working



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Abstract

This protocol is optimized for to stain free floating sections for fluorescent labelling. Please note than each step must be done on a shaker.



Day 1

- 1 Using a 12-well plate with buckets, wash sections 3×10min TBS
- 2 Depending on antibody of choice, do an antigen retrieval step.
Antigen retrieval in TBS:
10mM sodium citrate
0.05% Tween-20
- 3 Block in 5% serum in TBST for 1hour at RT (using approx 2mL per well)
- 4 Using approx 500uL per well, let sections wash in primary antibody solution in TBST + 1% serum overnight in  4 °C

Day 2

- 5 Wash sections 3×10min in TBST
- 6 Incubate in secondary antibody (1:1000 for Invitrogen-brand or 1:2000 for BioRad-brand in TBST + 1%serum) for 2 hours @ RT in the dark. Approx 2mL per well.
- 7 Wash 3×10min in TBS
- 8 Mount and allow slides to dry. Coverslip once sections are completely dried on the slide (.