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Immunohistochemistry for anti-GFP

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

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Abstract

Hnasko lab protocol for anti-GFP immunohistochemistry

Protocol:

1 Day 1

1.1 Wash with 1X PBS (Phosphate Buffered Saline, 10X solution, Fisher bioreagents, BP399-1) 5m
pH 7.4 00:05:00 3 times

1.2 Wash with 1X PBS containing 0.2% Triton X-100 (Sigma-Aldrich, X100-1L) (PBS-T). 5m
00:05:00 3 times

1.3 Blocking step with appropriate normal serum (use serum from the source species for the secondary antibodies to prevent nonspecific binding). 1h
Incubate with 4% NDS (Normal Donkey Serum, Jackson ImmunoResearch, 017-000-121) in PBS-T. 01:00:00 minimum at Room temperature

1.4 Incubate with primary antibodies in PBS-T with 4% NDS.
Overnight at 4 °C
Wrap well-plate air-tight with parafilm and cover with foil.
Rabbit anti-GFP polyclonal antibody, Invitrogen, A11122 (*Upon arrival: Diluted 1:1 in glycerol by adding same volume 100% glycerol, and stored at -20C). Dilution: 1:1000 to have a final dilution of 1:2000. Example: 1 µL anti-GFP + 999 µL PBS-T 4% NDS

2 Day 2

2.1 Wash with 1X PBS-T. 00:10:00 3 times 10m

2.2 Incubate with secondary antibodies in 1X PBS-T. 2h
02:00:00 at Room temperature
Cover well-plate with foil.
AlexaFluor 488 Donkey anti-rabbit, Jackson ImmunoResearch, 711-545-152 (*Upon arrival: Reconstituted with ~0.4 ml 50% glycerol and stored at -20C). Dilution: 1:400.
Example: 1 µL D@Rb488 + 399 µL PBS-T

2.3 Wash with 1X PBS. 00:10:00 3 times 10m

2.4 Mount on Slides (22-034-979, Fisherbrand). Takes practice!

2.5 Let dry on a slide holder (VWR, 48429-105). Cover from light

2.6 Rinse with ddH2O. Briefly dip slide in a 50mL falcon tube filled with ddH2O

2.7 Place back on slide holder and let dry covered from light

2.8 Apply ~ 120 µL of Fluoromount-G Fluorescent mounting medium (SouthernBiotech, 0100-01) with 0.5 µg/ml DAPI (Millipore, 10236276001) on dried slides and apply coverslip (2980-225, Corning)

2.9 Protect from light and dry overnight before imaging

Material:

3 Use a 24-well plate. Place a maximum of 12 sections / well. Use 1 mL / well for washes and blocking. Use from 500 µL to 750 µL / well for antibody incubations. Use fire-shaped blunted Pasteur glass pipette or thin brush to transfer section from 1 well to another. Place well plate on shaker, rocker, or belly dancer for all washes and incubation steps.

Note:

4 Discard any sections found stuck to side of wells

Samples:

5 30um-sections in 48 well-plates at 4 °C in 1X PBS with 0.01% Sodium Azide (Fisher Scientific,
BP922I-500) solution

Purpose:

6 GFP or YFP amplification. This protocol can also be applied to other epitopes/antibodies.