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Immunohistochemistry for anti-TH



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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-TH immunohistochemistry



Protocol:

1 Day 1

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

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

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

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.
Sheep anti-TH polyclonal antibody, Pel-Freez, P60101-150. Dilution: 1:1000.
Example:  1 µL anti-TH +  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.  02:00:00 at  Room temperature 2h

Cover well-plate with foil.
Alexa 647 Donkey anti-sheep, Jackson ImmunoResearch, 713-605-147 (*Upon arrival: dilute 1:1 with 100% glycerol for a final concentration of 50% glycerol and store at -20). Dilution: 1:400.
Example:  1 µL D@Sh647 +  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 μ L of Fluoromount-G Fluorescent mounting medium (SouthernBiotech, 0100-01) with 0.5 μ g/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 μ L to  750 μ L / 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 Visualization of TH/dopamine neurons. This protocol can also be applied to other epitopes/antibodies.