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# Immunostaining

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

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## Abstract

This protocol describes an immunofluorescence staining procedure with hydrogen peroxide quenching to reduce endogenous peroxidase activity, optimized for detection of TH and Myc/Cherry signals. Tissue sections are permeabilized with PBS-T, quenched with 3% $H_2O_2$ in the dark, and blocked with BSA before overnight incubation with primary antibodies against tyrosine hydroxylase (TH) and Myc. Fluorescent secondary antibodies and NeuroTrace are then applied for visualization, with thorough PBS-T washes between steps to minimize background. The protocol supports robust, specific labeling with reduced autofluorescence and background.

## Materials

**Solutions:**
**PBS-T Triton 0.2 % 150mL:** 300uL Triton in 150mL PBS

## Before start

**All washing steps are performed for 10min and on a shaker**

## Immunostaining: AOF-PD: myc/cherry, TH quench

1 **Washes** Wash 3x in PBS-T, 10min each (0.2% Triton in PBS)

2 **Quench** Incubate in 3% H2O2 in PBST for 2hrs at RT in the DARK! (x3) (can be done the day before and sections can be stored in PBS ON at 4degrees)

3 **Washes** Wash 3x in PBS-T, 10min each (0.2% Triton in PBS)

4 **Blocking I**ncubate in 3% BSA/PBS-T for 1 hr at room temperature.

5 **Primary Antibody** Incubate in primary antibody (diluted in blocking solution) overnight.

5.1 **Rabbit anti-TH Abcam, cat. Ab113, 1:1000**

5.2 **Mouse anti-Myc Cell Signaling, cat. 2276 1:1000**

6 **Washes** Wash 3x in PBS-T, 10min each

7 **Secondary Antibody** Incubate in secondary antibody (diluted in blocking solution) 1hr at room temperature.

7.1 **Donkey anti-rabbit 647 1:500**

7.2 **Donkey anti-mouse 488 1:1000**

7.3 **Neurotrace 455 (1:200)**

8 **Washes** Wash 3x in PBS-T, 10min each