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Fluorescent Immunolabelling for endogenous ChAT in mice striatum

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We use this protocol and it's working

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Abstract

This immunohistochemistry protocol has been designed and adjusted specifically for ChAT immunolabelling in the mice striatum

Materials

- Goat anti-ChAT antibody **Millipore Catalog #**AB144P
- Donkey anti-Goat IgG (H L) Cross-Adsorbed Secondary Antibody Alexa Fluor™ 488 **Thermo Fisher Scientific Catalog #**A-11055
- DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) **Thermo Fisher Catalog #**D1306
- Donkey serum **Sigma Aldrich Catalog #**S30-100ML

Solution

Tris Buffer Soaline Triton TBST - 2L
- 50mM Tris pH 7.5 – 100 mL of 1M stock
- 150 mM NaCl – 17.53 g
- Distilled Water – 1.9 L
- Triton 0.2 % - 4 mL

1 Select floating sections of mouse striatum (30um thick).

2 Wash: 3 x 00:10:00 gently on a shaker, in TBST at Room temperature . 10m

3 During the washes, prepare blocking solution: 10 % donkey serum (DS) in TBST.

4 Blocking step: Incubate the slides with blocking solution on the shaker for 01:00:00 at Room temperature . 1h

5 Before the end of the blocking step, prepare the solution with the primary antibody ChAT blocking solution (TBST+10% DS) + Goat Anti-Chat Ab ◊ dilution 1:200.

6 Primary antibody: incubate the slides with the blocking solution/Antibody on a shaker at 4 °C for 24:00:00 48:00:00 . 3d

7 Washes: 3 x 00:10:00 on a shaker, in TBST. 10m

8 During the washes, prepare the secondary Antibody solution
blocking solution (TBST+10% DS) + anti donkey anti goat Alexa Fluor 1:1000.

9 incubate the slides with Secondary antibody solution 01:00:00 at Room temperature . 1h

10 Washes: 2 times, for 00:10:00 in TBST. 10m

11 *Optional: DAPI staining*
During the washes, prepare the DAPI solution
DAPI (1 mg/ml). Dilution 1:10.000 in TBST.

12 Incubation the slides 00:10:00 with the DAPI solution. 10m

13 Washes: 2 times, for 00:10:00 each in TBST. 10m

14 Mounting on Superfrost+ slides.

15 Coverslip: mounted with prolong anti-fade reagent (Invitrogen).