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Fluorescent Immunolabelling for endogenous ChAT in mice striatum

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Protocol status: Working

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 **Merck Millipore (EMD 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 **Merck MilliporeSigma (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).