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Immunofluorescence on Free-Floating Brain Sections

In 1 collection

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Cristian González-Cabrera[1], Pablo Henny[2]

[1]LIN; [2]Universida de Chile

Cristian González-Cabrera
Leibniz Institute für Neuroniologie

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

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Abstract

This protocol describes immunofluorescence staining of free-floating coronal brain sections for neurochemical characterization of juxtacellularly labeled neurons. Antigen retrieval was performed selectively for VGAT and VGluT2 staining. Confocal images were acquired using identical acquisition settings across experimental conditions.

Materials

Solutions
- Phosphate-buffered saline (PBS), 0.01 M.
- Sodium citrate buffer, 0.1 M, pH 6.0.
- Blocking solution: PBS with 3 percent normal horse serum and 0.3 percent Triton X-100.
- Fluorescence mounting medium.

Primary Antibodies
- Guinea pig anti-Tyrosine Hydroxylase (TH), 1:5000, Synaptic Systems.
- Rabbit anti-VGluT2, 1:2000, Synaptic Systems.
- Rabbit anti-VGAT, 1:5000, Synaptic Systems.

Secondary Antibodies
- Species-appropriate Alexa Fluor 488 or Alexa Fluor 635 conjugates.
- Dilution 1:1000, Jackson ImmunoResearch.

Tissue Preparation
- Free-floating coronal sections.
- Section thickness: 40 µm.

Procedure

1 Rinse sections 3 times in PBS.
5 min per wash.

2 **Antigen retrieval**
Incubate sections in 0.1 M sodium citrate buffer, pH 6.0.
80 °C for 30 min.
Rinse 3 times in PBS.
Note: Antigen retrieval was performed only for VGAT and VGluT2 staining.

3 Incubate sections for 1 h at room temperature.
Blocking buffer: PBS + 3 percent normal horse serum + 0.3 percent Triton X-100.

4 Incubate overnight at 4 °C.
Primary antibodies diluted in blocking buffer.

5 Rinse sections 3 times in PBS.

6 Incubate 2 h at room temperature.
Protect from light.
Secondary antibodies diluted 1:1000.

7 Rinse sections 3 times in PBS.
Mount onto glass slides.
Coverslip using fluorescence mounting medium.

Imaging

8 Confocal microscopy.
Identical laser power and detector settings across conditions.
Settings kept constant within antibody sets.

Critical Steps

9 Perform antigen retrieval only for VGAT and VGluT2.
Maintain consistent antibody dilutions across experiments.
Protect fluorescent samples from light during and after secondary incubation.
Use identical confocal settings for comparisons.

Expected Outcome

10 Successful staining yields clear neurochemical identification of labeled neurons, allowing classification based on TH, VGluT2, or VGAT expression.