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IHC_cFOS+Parvalbumin

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We use this protocol and it's working

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Abstract

Immunohistochemistry protocol for c-FOS and Parvalbumin co-staining of mouse brain tissue slices (30 microns thick).

Guidelines

All shaker incubations are done at 200 rpm speed.

Staining

1 Antigen Retrieval
Remove PBS from wells. Add 500ul 0.3% citrate buffer into each well.

1.1 Incubate at RT on shaker for 30 min
Incubate at 65 °C for 45 min
Cool down on the bench for 20 min

2 3X PBS Washes

2.1 Remove citrate buffer from wells and replace with 500ul 1X PBS (or use a dropper pipette and fill the well halfway)

2.2 Incubate at RT on shaker for 15 min
remove PBS and replace with new 1X PBS, repeat two more times

3 Block Non-Specific Signal
Make enough: 10% NGS (Vector Labs, #S-1000-20) in 0.3% triton-X PBS for all samples (500ul per well)

3.1 Remove PBS and replace with blocking reagent 500ul per well.

3.2 Incubate on shaker RT for 60min.

4 Primary Antibody
Make enough: mouse anti-parv (1:1000, Sigma-Aldrich, #P3088) and rabbi anti-cfos (1:1000, cell Signaling, #2250S) in 1% NGS (Vector Labs, #S-1000-20) in 0.3% triton-X PBS

4.1 Replace the blocking reagent with primary antibody mix, 500 ul per well, and incubate at
4 °C
Overnight

5 DAY 2

3X PBS Washes

5.1 Remove primary Ab mix from wells and replace with 500ul 1X PBS (or use a dropper pipette and fill the well halfway)

5.2 Incubate at RT on shaker for 15 min repeat two more times

6 Secondary Antibody
Make enough: goat anti-mouse AF-555 (1:500, Invitrogen, #A-21422) and biotin goat anti-rabbit (1:1000, Vector Labs, #PK-6101) in 1% NGS (Vector Labs, #S-1000-20) in 0.3% triton-X PBS

6.1 Remove PBS from wells and replace with 500ul Secondary antibody mix. Cover with aluminum foil. Note: secondary antibodies are light sensitive, so from this point on the plate MUST be covered from light as much as possible.

Incubate at RT on shaker for 120 min.

7 3X PBS washes as described in Step 5 and 5.1 with the plate covered

8 Tertiary Antibody (for cFOS only)
make enough: AF-488 streptavidin conjugated (1:1000, Thermo Fisher, #S11223) in 1% NGS (Vector Labs, #S-1000-20) in 0.3% triton-X PBS

8.1 Replace PBS in wells with 500uL of tertiary antibody solution, cover the plate with aluminum foil, and incubate at RT on shaker for 120 min.

9 3X PBS washes as described in step 5 and 5.1 with the plated covered

10 Mounting with medium with and without DAPI