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Immunohistochemistry against p62 and LBP110

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Dennis de Bakker[1]

[1]Leibniz Institute on Aging – Fritz Lipmann Institute (FLI)

Dennis de Bakker
Leibniz Institute on Aging – Fritz Lipmann Institute (FLI)

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

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Abstract

Immunohistochemistry staining co-labeling LBP110 and p62/SQSTM1 and DAPI.

Materials

Buffer PEM (pH 7.4):
80 mM PIPES (Roth 9156.3)
5 mM EGTA (AppliChem (A0878,0025))
1 mM MgCl2 (Merck A748033 703)

Buffer PEMTx:
PEM (described above) + 0.2% Triton X-100

Sodium citrate solution (10mM) (pH 6.0)
10mM sodium citrate
Adjust pH to 6 with 1M Citric Acid

Blocking buffer (to make 1mL, which is good for 2 slides):
890ul PEMTx
100ul Fetal Calf Serum
10ul DMSO

Antibodies:
- Mouse anti-p62/SQSTM1 (clone 2C11) (Novus Biologicals, catalog no. H00008878-M01). Used 1:500.
- Rabbit anti-LBP110. Used 1:100. Antibody produced as described in: Kibbey, M. C., Jucker, M., Weeks, B. S., Neve, R. L., van Nostrandi, W. E., & Kleinman, H. K. (1993). f8-Amyloid precursor protein binds to the neurite-promoting IKVAV site of laminin. In *Proc. Natl. Acad. Sci. USA* (Vol. 90).
- Anti-mouse Alexa555 (used 1:500) (Invitrogen)
- Anti-rabbit Alexa647 (used 1:500) (Invitrogen)

Tissue preparation

1 Day 1: Whole mouse brain (2 years old, male) was fixed overnight in 4%PFA.

2 Day 2: Brain was washed 3x 5' in PBS + 4% Sucrose

3 Day2: Brain was incubated overnight in PBS + 30% Sucrose

4 Day3: Brain was embedded into optimal cutting medium (OCT)

5 Day3: Brain was cut with a cryostat in 10um thick sections and stored at -80C

Antibody staining protocol day 1 15m

6 Slides with brain tissue sections were thawed 00:15:00 Room temperature 15m

7 Add slides to glass coplin Jar.

8 Wash 3x 00:10:00 in PEMTx 10m

9 Move slides to plastic coplin jar for antigen retrieval

10 Perform antigen retrieval 00:15:00 85 °C 15m

11 Let plastic coplin jar cool down to room temperature 00:20:00 20m

12 Place slides back in original glass complin jar

13 Wash 2x 00:10:00 in PEMTx 10m

14 Place slides horizontally in box humidified with MQ. Use hydrophobic pen to outline tissue sections.

15 Add blocking buffer to the slides and incubate 01:00:00 1h

16 Add antibody solution (blocking buffer + 1:100 LBP110 + 1:100 p62) and incubate Overnight 4 °C

Antibody staining protocol day 2 15m

17 Wash slides 4x 00:15:00 15m

18 Incubate with secondary antibody solution in humidified box (PEMTx + 2% BSA + 1:500 secondary antibodies + 1:1000 DAPI) for 01:00:00 1h

19 Place slides back in coplin jar (keep in dark)

20 Wash 3x 00:10:00 in PEMTx 10m

21 Wash 2x 00:07:00 in PEM 7m

22 Mount slides using Prolong Gold mounting medium and store at 4 °C

Protocol references

LBP110 antibody: Kibbey, M. C., Jucker, M., Weeks, B. S., Neve, R. L., van Nostrandi, W. E., & Kleinman, H. K. (1993). f8-Amyloid precursor protein binds to the neurite-promoting IKVAV site of laminin. In *Proc. Natl. Acad. Sci. USA* (Vol. 90).