

Jul 31, 2024

Immunohistochemistry against p62 and LBP110

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg32b47v25/v1>

Dennis de Bakker¹

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



Dennis de Bakker

Leibniz Institute on Aging – Fritz Lipmann Institute (FLI)

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kqdg32b47v25/v1>

Protocol Citation: Dennis de Bakker 2024. Immunohistochemistry against p62 and LBP110. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kqdg32b47v25/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: July 30, 2024



Last Modified: July 31, 2024

Protocol Integer ID: 104292

Keywords: Immunohistochemistry, mouse hippocampus, cryosections, lbp110 immunohistochemistry, immunohistochemistry against p62, immunohistochemistry, p62, staining co

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 Nostrand, 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 3× 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 Nostrand, 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).