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Validation of phosphor-ERM Antibody Specificity in WT and LRRK2 G2019S Knock-In(ki/ki) Mouse Brain Sections

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

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Disclaimer

Abstract

To confirm the specificity of the antibody used to detect phosphorylated ERM (pERM) in immunostaining by using Lambda protein phosphatase treatment on brain sections from both wild-type (WT) and LRRK2 G2019Ski/mice. Lambda protein phosphatase dephosphorylates proteins, which should eliminate phospho-ERM staining if the antibody is specific to the phosphorylated form of ERM.

Objective

1 To confirm the specificity of the antibody used to detect phosphorylated ERM (pERM) in immunostaining by using Lambda protein phosphatase treatment on brain sections from both wild-type (WT) and LRRK2 G2019S$^{ki/ki}$ mice. Lambda phosphatase dephosphorylates proteins, which should eliminate phospho-ERM staining if the antibody is specific to the phosphorylated ERM.

Materials

2 WT and LRRK2 G2019S$^{ki/ki}$ mouse brain sections

3 Reagents

3.1 Rabbit Phospho-ERM antibody: https://www.cellsignal.com/products/primary-antibodies/phospho-ezrin-thr567-radixin-thr564-moesin-thr558-antibody/3141

3.2 Lambda Protein phosphatase enzyme and buffer: https://www.neb.com/en-us/products/p0753-lambda-protein-phosphatase-lambda-pp?srsltid=AfmBOoqA4jK4IeWqMweJTU25CO0o4dyd5r3o11Mx9aN49mr1KlCzIcXC

Preparation of Brain Sections

4 Harvest brain tissues from WT and LRRK2 G2019Ski/ki mice (age matched)

5 Fix brain tissues in 4% paraformaldehyde (PFA) for 24 hours at 4 degrees.

6 Cryoprotect tissues in 30% sucrose solution until fully equilibrated

7 Embed and section brains using a cryostat to obtain 30um thick coronal sections, and store sections at -20 degrees

Treatment Conditions

8 Divide brain sections from both WT and LRRK2 G2019Ski/ki mice into two groups

8.1 Lambda Protein Phosphatase Treatment Group: Treat sections with Lambda Protein phosphatase to dephosphorylate phospho-ERM

8.2 Control Group: Incubate sections under identical conditions but without Lambda Protein Phosphatase

Lambda Protein Phosphatase Treatment

9 Prepare Lambda Protein Phosphatase solution according to manufacturer instructions. https://www.neb.com/en-us/products/p0753-lambda-protein-phosphatase-lambda-pp?srsltid=AfmBOoqA4jK4leWqMweJTU25CO0o4dyd5r3o11Mx9aN49mr1KlCzIcXC

10 Incubate sections from the treatment group in the Lambda Protein phosphatase solution for 2 hours at 30°C.

11 Wash sections thoroughly with TBS to remove residual enzymes.

Immunostaining

12 Block all sections in blocking solution (e.g., 5% normal goat serum and 0.3% Triton X-100 in PBS) for 1 hour at room temperature.

13 Incubate sections overnight at 4°C with the primary antibody targeting pERM at 1:100.

14 Wash sections with TBS three times.

15 Incubate sections overnight at 4°C with the secondary antibody

16 Wash with TBS and mount with STED mounting medium.

Imaging and Data Acquisition

17 Image all sections using Olympus FV3000, Capture regions of interest within each section