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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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We use this protocol and it's working

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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?srsId=AfmBOoqA4jK4leWqMweJTU25CO0o4dyd5r3o11Mx9aN49mr1KlCzlcXC>

Preparation of Brain Sections

- 4 Harvest brain tissues from WT and LRRK2 G2019S^{ki/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 G2019S^{ki/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?srltid=AfmBOoqA4jK4leWqMweJTU25CO0o4dyd5r3o11Mx9aN49mr1KlCzlcXC>
- 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