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Protocol for α-synuclein (αSyn) proximity ligation assay (PLA) for detecting α-synuclein oligomers in rodents

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We use this protocol and it's working

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Abstract

Protocol for α-synuclein (αSyn) proximity ligation assay (PLA) for detecting α-synuclein oligomers in rodents.

Troubleshooting

Antibody conjugation with Duolink Probes

1 Add 2 µL of Conjugation buffer from the Duolink PLUS (Merck, DUO92009-1KT) or Duolink MINUS (Merck, DUO92010-1KT) probes to rabbit anti-alpha-synuclein polyclonal antibody (Merck, AB5038P).
NOTE: Consider that primary antibody should be made in goat, rabbit or mouse and should be in amine-free buffer at a concentration of 1mg/mL.

2 Mix by gently pipetting.

3 Transfer the antibody solution to one vial of lyophilized oligonucleotides (PLUS or MINUS).

4 Incubate at room temperature overnight.

5 Add 2 µL of Stop Reagent to the reaction.

6 Incubate at room temperature for 30 minutes.

7 Add 24 µL of Storage Solution and store it at 4ºC.

PLA processing

8 Rat formalin-fixed paraffin embedded tissue blocks from the substantia nigra sectioned at 5µm were incubated in the oven at 60°C for 30 minutes to melt the paraffin.

9 To remove the paraffin, sections were submerged in Xylene (Panreac Applichem, 211769.2714) for 3 × 3 minutes, followed by rehydration in decreasing ethanol concentrations (100% ethanol for 2 × 5 minutes, 95% ethanol for 2 × 5, 70% ethanol for 2×5 minutes) and distilled H2O for 5 minutes.

10 To unmask epitopes, sections were submerged in citrate buffer 10mM, pH6 and incubated in water bath at 95ºC for 20 minutes followed by 20 minutes at room temperature.

11 Slides were then washed 2 × 5 minutes in PBS 1x-triton 0,5% buffer followed by 1 × 5 minutes in PBS 1x buffer.

12 For the blocking, tissue sections were dried and circled with a hydrophobic pen (Vector laboratories, NC9545623). Tissue sections were then incubated in Duolink blocking solution from Duolink PLUS or MINUS (1 – 2 drops/section).

13 Conjugated primary antibodies (PLUS and MINUS) were diluted at 1:500 with the antibody diluent. Tissue sections were then incubated with the primary antibodies 1 overnight at 4ºC.

14 Slides were washed 2 × 5 minutes in 1x Wash Buffer A (Merck, DUO82049-4L).

15 For ligation, 5x Duolink ligation buffer was diluted in 1:5 high purity water and then mixed to create the ligation buffer. Ligase was then diluted in ligation buffer at 1:40. Tissue sections were then incubated 30 minutes at 37ºC.
NOTE: Wait to add the ligase until immediately prior to addition to the sample. Make sure ligation buffer is completely thawed and mixed well prior to usage.

16 Slides were washed 2 × 5 minutes in 1x Wash Buffer A (Merck, DUO82049-4L).

17 For amplification, 5x Duolink amplification buffer was diluted in 1:5 high purity water and then mixed to create the amplification buffer. Polymerase was then diluted in amplification buffer at 1:80. Tissue sections were then incubated 100 minutes at 37ºC.
NOTE: Wait to add the polymerase until immediately prior to addition to the sample. The Amplification buffer is light-sensitive. Protect all solutions containing buffer from light.

18 For final washes, slides were washed 2 × 10 minutes 1x Wash Buffer B (Merck, DUO82049-4L) and in 0.01x Wash B for 1 minute.

19 Tissue sections were then incubated with DAPI (Thermo Fisher Scientific, H3570) at 1:5000 for 10 minutes at room temperature.

20 Slides were mounted with DAKO (Merck, F4680) and stored at 4ºC.