



JAN 29, 2024

OPEN ACCESS



DOI:

[dx.doi.org/10.17504/protocols.io.bp2l6x6eklqe/v1](https://dx.doi.org/10.17504/protocols.io.bp2l6x6eklqe/v1)

**Protocol Citation:** alba.nicolau, Thais Cuadros, Miquel Vila 2024. Protocol for  $\alpha$ -synuclein ( $\alpha$ Syn) proximity ligation assay (PLA) for detecting  $\alpha$ -synuclein oligomers in rodents. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bp2l6x6eklqe/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

## 🌐 Protocol for $\alpha$ -synuclein ( $\alpha$ Syn) proximity ligation assay (PLA) for detecting $\alpha$ -synuclein oligomers in rodents

alba.nicolau<sup>1,2</sup>, Thais Cuadros<sup>1,2</sup>, Miquel Vila<sup>1,2,3,4</sup>

<sup>1</sup>Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute (VHIR)-Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED), 08035 Barcelona, Spain;

<sup>2</sup>Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815;

<sup>3</sup>Institute of Neurosciences, Autonomous University of Barcelona (INc-UAB), Bellaterra, Barcelona, Spain;

<sup>4</sup>Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

Vilalab Public



Miquel Vila

### ABSTRACT

Protocol for  $\alpha$ -synuclein ( $\alpha$ Syn) proximity ligation assay (PLA) for detecting  $\alpha$ -synuclein oligomers in rodents.

Created: Jan 29, 2024

Last Modified: Jan 29, 2024

PROTOCOL integer ID: 94296

**Funders Acknowledgement:**

Aligning Science Across

Parkinson's (ASAP)

Grant ID: ASAP-020505

## Antibody conjugation with Duolink Probes

- 1 Add 2  $\mu$ L 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  $\mu$ L of Stop Reagent to the reaction.
- 6 Incubate at room temperature for 30 minutes.

- 7 Add 24  $\mu$ L of Storage Solution and store it at 4°C.

## PLA processing

- 8 Rat formalin-fixed paraffin embedded tissue blocks from the substantia nigra sectioned at 5 $\mu$ m 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 x 3 minutes, followed by rehydration in decreasing ethanol concentrations (100% ethanol for 2 x 5 minutes, 95% ethanol for 2 x 5, 70% ethanol for 2x5 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 95°C for 20 minutes followed by 20 minutes at room temperature.
- 11 Slides were then washed 2 x 5 minutes in PBS 1x-triton 0,5% buffer followed by 1 x 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 4°C.

- 14 Slides were washed 2 x 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 37°C.  
*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 x 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 37°C.  
*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 x 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 4°C.