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Indirect Proximity Ligation Assay (PLA) - Fluorescence

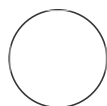
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ABSTRACT

Indirect Proximity Ligation Assay (PLA) is a powerful molecular technique used to detect and visualize protein-protein interactions, protein modifications, and protein complex formations within cells or tissues. This method is based on the principles of proximity-dependent ligation and utilizes specific antibodies to detect the nitration of proteins on free-floating brain sections. Here we describe the PLA protocol that we routinely use in our laboratory to detect nitrated alpha-synuclein and nitration of mitochondrial enzymes such as SOD2 and the mitochondrial complex 1 subunit NDUFB8.

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

MATERIALS

Created: Aug 02, 2023

Blocking solution: Vector Lab SP-6000-100

Last Modified: Aug 02, 2023

Duolink In Situ Wash Buffers, Fluorescence: DUO82049-20L

PROTOCOL integer ID: 85854

Duolink InSitu PLA probe anti-rabbit PLUS kit: DUO92002-100RXN

Duolink InSitu PLA probe anti-mouse MINUS kit: DUO92004-100RXN

Keywords: post-translational modification, alpha-synuclein, oxidative stress

Duolink In Situ Detection Reagents Red: DUO92008-100RXN

Duolink *In Situ* Mounting Medium with DAPI: DUO82040-5ML

Antibodies:


mouse anti-3-NT: 1:250; ab61392, Abcam


rabbit anti-human alpha-synuclein (clone MJFR1): 1:4000, ab138501, Abcam

rabbit anti-SOD1: 1:1000; ADI-SOD-110, Enzo Life Sciences

rabbit anti-NDUFB8: 1:300; 14794, Proteintech

Day 1







- 1 Pick 35um cut brain sections and transfer them to  1.5 mL Eppendorf tubes:
Note: all incubation and wash steps are performed by shaking Eppendorf tubes at 250rpm (e.g., thermomixer)

- 2 Wash 2x  00:05:00 with Tris-HCl

5m




- 3 Wash 3x  00:05:00 with wash buffer A (see materials)

5m

- 4 **Antigen-retrieval** with citrate buffer (+tween, pH = 6) at 95°C for  00:04:00 4m
Note: Heat the solution to 95°C before adding on the samples
- 5 Wash 3x  00:05:00 with wash buffer A 5m
- 6 **Blocking:** Incubate in Duolink Blocking Solution ( 300 µL) at 37°C for  01:00:00 1h
- 7 **Primary Antibody Incubation:** Dilute antibodies in Duolink Antibody Diluent ( 200 µL) and incubate  Overnight room temperature. 5m
for nitrated alpha-synuclein: mouse anti-3-NT and rabbit anti-h-alpha-synuclein
for nitrated SOD2: mouse anti-3-NT and rabbit anti-SOD2
for nitrated NDUFB8: mouse anti-3-NT and rabbit anti-NDUFB8
see materials for dilutions and catalog numbers

Day 2

10m

- 8 Wash 3x  00:10:00 with wash buffer A 10m
- 9 **PLA probe incubation:** 1h 30m
Prepare anti-mouse MINUS and anti-rabbit PLUS probes following manufacturer's instructions, add solution on sections and incubate at 37°C for  01:30:00
- 10 Wash 3x  00:10:00 with wash buffer A 10m

- 11 Ligation:** 1h 15m
Dilute the Duolink Ligation Buffer 1:5 in high-purity water
Add the ligase (diluted 1:40) just before incubation (keep cold on freezer block!)
add the ligation solution on sections and incubate at 37°C for 01:15:00
- 12** Wash 3x 00:05:00 with wash buffer A 5m
- 13 Amplification:** This step is light-sensitive! incubation is performed using aluminum foil to protect samples from light. 2h 30m
Dilute the Duolink Amplification Buffer (red) 1:5 in high-purity water
Add the polymerase (diluted 1:80) just before incubation (keep cold on freezer block!)
Add the amplification solution to the sections and incubate at 37°C for 02:30:00
- 14** Wash 2x 00:05:00 with wash buffer B 6m
Wash 1x 00:01:00 with wash 0.1x buffer B
- 15 Additional immunohistochemical staining (optional):** if immunohistochemical counter-staining is going to be performed it is important to avoid the use of detergents in wash buffers and perform long incubations in cold.
- 16** Mount the samples on slides and let them air-dry for 5 mins
- 17** Coverslip samples with Duolink *In Situ* Mounting Medium with DAPI, store samples in the dark at 4°C