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# 1 Indirect Proximity Ligation Assay (PLA) - Fluoresence

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Ayse Ulusoy<sup>1</sup>, Rita Pinto-Costa<sup>1</sup>, Angela Rollar<sup>1</sup>, Donato Di Monte<sup>1</sup>



### Ayse Ulusoy

German Center for Neurodegenerative Diseases

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We use this protocol and it's working

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**Keywords:** post-translational modification, alpha-synuclein, oxidative stress, ASAPCRN, fluoresence indirect proximity ligation assay, indirect proximity ligation assay, nitration of mitochondrial enzyme, nitration of protein, synuclein, mitochondrial enzyme, protein, subunit ndufb8, protein complex formations within cell, protein modification, protein interaction, dependent ligation, nitrated alpha, powerful molecular technique, specific antibody

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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.



### **Materials**

Blocking solution: Vector Lab SP-6000-100

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

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

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

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

# **Troubleshooting**



## Day 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)
- Wash 2x 00:05:00 with Tris-HCI

5m

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 Note: Heat the solution to 95°C before adding on the samples

4m

Wash 3x 00:05:00 with wash buffer A

5m

6 **Blocking:** Incubate in Duolink Blocking Solution ( Δ 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

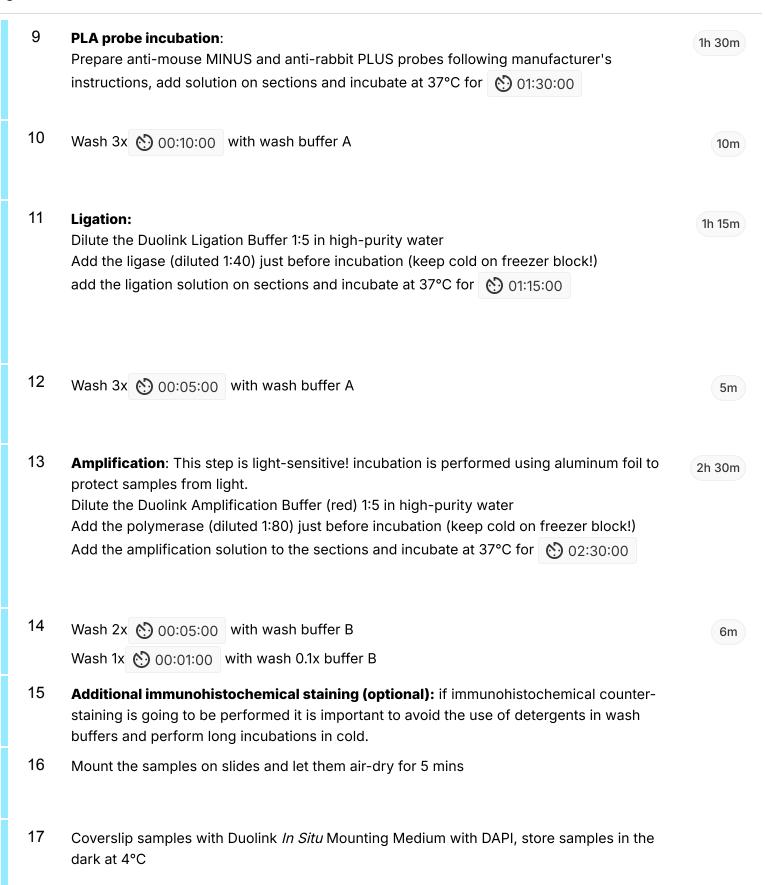
see materials for dilutions and catalog numbers

# Day 2

10m

8 Wash 3x 👏 00:10:00 with wash buffer A

10m





## **Protocol references**

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