

## Proximity Ligation Assay

### Manufacturer Supplied Protocol

## Fix and permeabilize the samples as follows:

- Tissue slices: Use 0.5% Triton X-100 in PBS for 10 minutes.
- Cells: Use 0.2% Triton X-100 in PBS for 5 minutes.
- Wash the samples three times with PBS.

## Blocking and antibody incubation

- Incubate the samples with a blocking solution for 1 hour at 37°C in a humidified chamber.
- Prepare the primary antibodies in antibody diluent and incubate the samples overnight at 4°C.

## Secondary Antibody Staining

- Wash the samples twice with Buffer A for 5 minutes each.
- Incubate the samples with PLA probes (anti-mouse MINUS and anti-rabbit PLUS) prepared in antibody diluent for 1 hour at 37°C.

## PLA reaction

- Wash the samples twice with Buffer A for 5 minutes each.
- Perform the ligation step by incubating the samples with ligase enzyme diluted in ligation buffer for 30 minutes at 37°C in a humidified chamber.
- Wash the samples twice with Buffer A for 5 minutes each.
- Incubate the samples with polymerase enzyme diluted in amplification buffer for 100 minutes at 37°C in a humidified chamber.
- Wash the samples twice with Buffer B for 10 minutes each.
- Perform a final wash with 0.01% Buffer B for 1 minute.

## Mounting

- Mount the slides with Duolink in situ mounting medium containing DAPI.
- Imaging and Storage
- Wait 15 minutes before imaging using a fluorescence or confocal microscope with at least a 20x objective.
- After imaging, store the slides:
- In the dark at 4°C for up to 4 days.
- Or at -20°C for up to 6 months.

## Control Experiments

Perform negative control experiments by incubating the samples with only one or none of the primary antibodies before adding the PLA probes.

## Material

The kit is stored all over RT, 4C and -20C

## Room Temp

- Buffer A and buffer B powder in chemical cabinet, genomics “D15”

## 4C

4F4 fridge in freezer room with glass room, box in the middle shelf filled with smaller boxes, “Duolink” original boxes contain:

- PLA probes plus rabbit
- PLA probes minus mouse
- Blocking
- Antibody diluent
- Mounting media

## -20C 20F2 B7 freezer room

- Ligation stock (5x)
- Ligase (1U/ul)
- Amplification Stock Red(5x)
- Amplification Stock Green(5x)
- Polymerase (10U/uL)