Duolink® In Situ

SHORT INSTRUCTIONS – FLUORESCENCE

1. BLOCKING

Add blocking solution to samples.
Incubate.

- *Remove block*

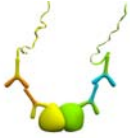
2. PRIMARY ANTIBODIES

Dilute the primary antibodies in appropriate buffer and apply to samples.
Incubate.

- *Wash in suitable buffer for 2 × 5 min*

3. PLA® PROBES

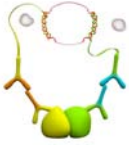
Dilute the two PLA probes 1:5 in appropriate buffer and apply to samples.
Incubate for 60 min at +37°C.



- *Wash in 1x Wash Buffer A for 2 × 5 min*

4. LIGATION

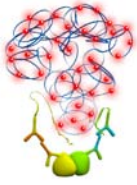
Dilute the Ligation stock 1:5 in H_2O. Dilute the Ligase at 1:40 in the solution and apply the mix to samples.
Incubate for 30 min at +37°C.



- *Wash in 1x Wash Buffer A for 2 × 2 min*

5. AMPLIFICATION

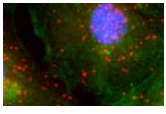
Dilute the Amplification stock 1:5 in H_2O. Dilute the Polymerase at 1:80 in the solution and apply the mix to samples.
Incubate for 100 min at +37°C.



- *Wash in 1x Wash Buffer B for 2 × 10 min*
- *Wash in 0.01x Wash Buffer B for 1 min*

6. PREPARATION FOR IMAGING

Mount the samples with Duolink In Situ Mounting Medium with DAPI, wait for 15 min, and analyze in a fluorescence or confocal microscope.



Note: *Use open droplet reactions without a cover slip and perform all incubations in a humidity chamber.*
Use volumes corresponding to your reaction area, see the Reaction Volume Guide (# 0062).
Use a freezing block when removing the enzymes from the freezer (-20°C).
Washing should be done in a minimum volume of 70 ml on a shaker with gentle orbital shaking.

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