Duolink™ in situ PLA protocol

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 (# 80520).



1. Blocking

Add blocking solution to sample

Incubate

Remove block



2. Binding of Primary Antibodies

Dilute the primary antibodies in appropriate buffer and apply to sample

Incubate

Wash



3. Binding of PLA probes

Dilute the PLA probes 1:5 in appropriate buffer and apply to sample

Incubate at 37°C for 120 minutes

Wash



4. Hybridization

Dilute the Hybridization stock 1:5 in H₂O and apply to sample

Incubate at 37°C for 15 minutes

Wash in TBS-T 1 min



5. Ligation

Dilute the Ligation stock 1:5 in H₂O, add Ligase at a 1:40 dilution, and apply mix to sample

Incubate at 37°C for 15 minutes

Wash in TBS-T 2 x 2 min



6. Amplification

Dilute the Amplification stock 1:5 in H₂O, add Polymerase at a 1:80 dilution, and apply mix to sample

Incubate at 37°C for 90 minutes

Wash in TBS-T 2 x 2 min

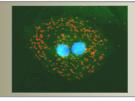


7. Detection

Dilute the Detection stock 1:5 in H₂O and apply to sample

Incubate at 37°C for 60 minutes

Wash in SSC series Final wash in 70% EtOH



8. Mount and analyze

Mount the sample with Duolink Mounting Medium and analyze in fluorescence microscope