SIGMA-ALDRICH®

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Product Information

Duolink® flowPLA Detection Kit - Green

Catalog Number **DUO94002**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Duolink® flowPLA Detection Kit - Green contains all the necessary Duolink PLA® reagents to perform the amplification and detection of bound PLA probes by flow cytometry. The detection oligonucleotides contain a fluorophore (λ_{ex} = 495 nm/λ_{em} = 527 nm).

Experiments conducted using Duolink flowPLA reagents can detect protein interactions, protein expression levels, and post-translational modifications at the single molecule level in fixed, suspended cells.

Components

Sufficient components are provided for 40 tests, based on 100 µL total reaction volume covering 100,000 cells.

5× Ligation Buffer – Contains oligonucleotides that hybridize to the PLA probes and all components needed for ligation except the ligase.
DUO82009-40 TST 800 µL

Ligase (1 unit/µL)
DUO82027 100 µL

Polymerase (10 units/µL)
DUO82028 50 µL

5× Amplification Buffer – Contains all components needed for rolling-circle amplification (RCA) except the polymerase.
DUO82050-40 TST 800 µL

5× flowPLA Detection Solution Green – Contains oligonucleotides labeled with a fluorophore that hybridize to the RCA product.
DUO84002-40 TST 800 µL

Reagents and Equipment Required but Not Provided.

To perform a complete Duolink flowPLA experiment, one will need two primary antibodies (IHC or ICC/IF validated) that recognize two target epitopes. Additional reagents include a pair of PLA probes (one 100RXN PLUS and one 100RXN MINUS) and flowPLA detection reagents of choice. Recommended reagents include Duolink Wash Buffers and PBS.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Thaw the 5× Ligation, 5× Amplification, and 5x flowPLA Detection Green buffers at room temperature and vortex before use. Dilute the required volumes of each 5× solution 5-fold with ultrapure water **immediately before use**. **Do not store diluted reagents.**
Note: The 5× Ligation Buffer contains DTT that may precipitate at –20 °C. Make sure the DTT is completely dissolved and vortexed before use.

The Duolink **Detection solutions** are **light-sensitive. Protect from light.**

The ligase and polymerase enzymes should be kept cold (–20 °C) at all times; use a freezing block when removing them from the freezer. Quick spin the vial before pipetting. Add the enzyme to the appropriate reaction mix **immediately before use**. Vortex the mix after addition of enzyme. **Do not store diluted reagents**.

Storage/Stability

Store the flowPLA reaction components at –20 °C. The enzymes should be kept cold (–20 °C) at all times, use a freezing block when removing them from the freezer.

Procedure
The experimental procedures for Duolink PLA Flow Cytometry application can be found at sigma.com/duolink-flow.

Note: Duolink PLA reagent volumes are based on a 40 μL reaction volume for a 1 cm^2 sample on a microscope slide or a 100 μL reaction volume at ~1,000 cells/μL for flow cytometry. However, volumes may need to be adjusted according to the sample size or number of cells of the sample.

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