3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Duolink® flowPLA Detection Kit – Violet
Duolink PLA kit for Flow Cytometry
with Violet Detection

Product Number **DUO94005**

Product Description

Duolink® flowPLA Detection Kit - Violet 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} = 390 nm/λ_{em} = 476 nm), which may be excited using the 405 nm violet laser line.

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× Detection Solution Violet – Contains oligonucleotides labeled with a fluorophore that hybridize to the RCA product.
DUO84005-40 TST 800 µL

Reagents 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 PLUS and one 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 5× Detection Violet 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.** The Duolink Detection solutions are light-sensitive. **Protect from light.**

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 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.

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

This product is coved by several patents and patent applications including US 6,511,809, US 6,558,928, US 6,878,515, US 7,074,564, US 5,665,539, and related US and foreign patents, including Japanese Patent No. 5653964.

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