

Product Information

Duolink® flowPLA Detection Kit - Far Red

Catalog Number **DUO94004**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

Duolink® flowPLA Detection Kit – Far Red 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 ($\lambda_{\text{ex}} = 644\text{ nm}/\lambda_{\text{em}} = 669\text{ nm}$).

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 \times 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 \times Amplification Buffer – Contains all components needed for rolling-circle amplification (RCA) except the polymerase. DUO82050-40 TST	800 μL
5 \times flowPLA Detection Solution Far Red – Contains oligonucleotides labeled with a fluorophore that hybridize to the RCA product. DUO84004-40 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 PLUS and one MINUS) and flowPLA detection reagents of choice. Recommended reagents include Duolink Wash Buffers and PBS.

Precautions and Disclaimer

This product is 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 \times Ligation, 5 \times Amplification, and 5 \times flowPLA Detection Far Red buffers at room temperature and vortex before use. Dilute the required volumes of each 5 \times solution 5-fold with ultrapure water **immediately before use. Do not store diluted reagents.**

Note: The 5 \times Ligation Buffer contains DTT that may precipitate at $-20\text{ }^{\circ}\text{C}$. 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 ($-20\text{ }^{\circ}\text{C}$) 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 $-20\text{ }^{\circ}\text{C}$. The enzymes should be kept cold ($-20\text{ }^{\circ}\text{C}$) 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 $\sim 1,000$ cells/ μL for flow cytometry. However, volumes may need to be adjusted according to the sample size or number of cells of the sample.

This product is covered 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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