Technical Bulletin

Duolink® flowPLA Detection Kit - Orange

DUO94003

Storage Temperature –20 °C

Product Description

Duolink® flowPLA Detection Kit - Orange 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} = 554 nm/ λ_{em} = 576 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.

- 5x Ligation Buffer:
  Contains oligonucleotides that hybridize to the PLA probes and all components needed for ligation except the ligase. Cat. No. DUO82009-40 TST (800 µL)
- Ligase (1 unit/µL) Cat. No. DUO82027 (100 µL)
- Polymerase (10 units/µL) Cat. No. DUO82028 (50 µL)
- 5x Amplification Buffer:
  Contains all components needed for rolling-circle amplification (RCA) except the polymerase. Cat. No. DUO82050-40 TST (800 µL)
- 5x flowPLA Detection Solution Orange:
  Contains oligonucleotides labeled with a fluorophore that hybridize to the RCA product. Cat. No. DUO84023-40 TST 800 µL

Reagents and Equipment Required

(not included)

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 5x Ligation, 5x Amplification, and 5x flowPLA Detection Orange buffers at room temperature and vortex before use. Dilute the required volumes of each 5x solution 5-fold with ultrapure water immediately before use. **Do not store diluted reagents.**

Note: The 5x 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 online at SigmaAldrich.com.

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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