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Product Information

Duolink™ flowPLA Multicolor Reagent Pack

Catalog Number: **DUO96001**

Storage Temperature: –20 °C

Product Description

Duolink™ flowPLA Multicolor Reagent Pack contains all the necessary reagents to perform the amplification and detection of bound custom PLA probes generated using Duolink™ PLA Multicolor Probemaker Kits.

To perform a Duolink™ PLA Multicolor experiment, custom PLA probes will need to be generated using the Duolink PLA Multicolor Probemaker Kits of choice (DUO96010-DUO96040). Following amplification, the 5x Multicolor PLA Detection buffer contains a combination of fluorophore-labeled detection oligonucleotides, which allows simultaneous detection of up to four protein events (protein interactions, post-translational modifications, and/or sensitive protein detection) within a fixed suspension cell sample by flow cytometry. The fluorophores are compatible with FITC (Green, λ_{ex} 490nm, λ_{em} 520nm), Cy™3 (Orange, λ_{ex} 554nm, λ_{em} 576nm), Texas Red® (Red, λ_{ex} 594nm, λ_{em} 624nm), or Cy™5 (FarRed, λ_{ex} 644nm, λ_{em} 669nm) filters for detection. Recommended additional reagents include Duolink™ Wash Buffer (DUO82047) and PBS (P3813).

Components

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

Product Description	Catalog Number	Quantity
5x Multicolor PLA Ligation Buffer - Contains oligonucleotides that hybridize to the PLA probes and all components needed for ligation except the ligase.	DUO86002	800 µL
Ligase (1 unit/µL)	DUO82025	100 µL
5× Amplification Buffer - Contains all components needed for rolling-circle amplification (RCA) except the polymerase.	DUO82050	800 µL
Polymerase (10 unit/µL)	DUO82026	50 µL
5x Multicolor flowPLA Detection Buffer – Contains oligonucleotides labeled with fluorophores that hybridize to the RCA products.	DUO86003	800 µL
1x Blocking Solution – For blocking of sample prior to Duolink™ PLA Multicolor staining.	DUO82007	8 mL
1x Probemaker PLA Probe Diluent – For diluting the PLA probes (conjugated antibody) to the final assay.	DUO82036	8 mL

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 practice.

Preparation Instructions

The 1x Blocking Solution and 1x Duolink Probemaker PLA Probe Diluent are supplied at ready-to-use concentrations. **After thawing, tighten the lid on the 1x Blocking Solution prior to use as the dropper may have become dislodged during freeze-thaw.** The 1x Blocking Solution and 1x Duolink Probemaker PLA Probe Diluent can be stored at +2-8 °C upon arrival.

Thaw the Multicolor PLA 5× Ligation, 5× Amplification, and 5x Detection 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 Multicolor flowPLA Detection solution is light-sensitive. Protect from light.**

Note: The Multicolor PLA 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
The Duolink™ flowPLA Multicolor Reagent Pack is shipped on dry ice. However, the 1x Blocking Solution and 1x Duolink™ Probemaker PLA Probe Diluent can be stored at +2-8 °C upon arrival. All other components should be stored at –20 °C.

Procedure
The Duolink™ PLA Probemaker Guide for Multicolor Detection and the Duolink™ flowPLA Multicolor Detection Protocol can be found at sigma.com/duolink. These documents describe the procedures for antibody conjugation and use of the custom PLA probes in Duolink™ flowPLA Multicolor experiments.

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