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Product Information

Duolink® PLA® Multicolor Reagent Pack

Catalog Number **DUO96000**
Storage Temperature –20 °C

Product Description

Duolink® PLA® 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 through DUO96040). Following amplification, the 5× 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 cell or tissue sample.

The fluorophores are compatible with the following detection filters:
FITC (Green, λ_{ex} = 490 nm/λ_{em} = 520 nm)
Cy™3 (Orange, λ_{ex} = 542 nm/λ_{em} = 562 nm),
Texas Red® (Red, λ_{ex} = 594 nm/λ_{em} = 624 nm)
Cy™5 (FarRed, λ_{ex} = 644 nm/λ_{em} = 669 nm)

Recommended additional reagents include Duolink Wash Buffers and Mounting Medium.

Components

Sufficient components are provided for the indicated number of reactions (30 or 100 RXN), based on 40 µL of the total reaction mixture covering 1 cm^2.

5× Multicolor PLA Ligation Buffer - Contains oligonucleotides that hybridize to the PLA probes and all components needed for ligation except the ligase.

30 RXN (DUO86002)	250 µL
100 RXN (DUO86002)	800 µL

Ligase (1 unit/µL)

30 RXN (DUO82027)	
100 RXN (DUO82025)	

5× Amplification Buffer - Contains all components needed for rolling-circle amplification (RCA) except the polymerase.

30 RXN (DUO82050)	250 µL
100 RXN (DUO82050)	800 µL

Polymerase (10 units/µL)

30 RXN (DUO82028)	
100 RXN (DUO82026)	

5× Multicolor PLA Detection Buffer – Contains oligonucleotides labeled with fluorophores that hybridize to the RCA products.

30 RXN (DUO86001)	250 µL
100 RXN (DUO86001)	800 µL

1× Blocking Solution – For blocking of sample prior to Duolink PLA Multicolor staining.

30 RXN (DUO82007)	4 mL
100 RXN (DUO82007)	8 mL

1× Probemaker PLA Probe Diluent – For diluting the PLA probes (conjugated antibody) to the final assay.

30 RXN (DUO82036)	5 mL
100 RXN (DUO82036)	8 mL

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

Thaw the Multicolor PLA 5× Ligation, 5× Amplification, and 5× 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 PLA 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 the enzyme. **Do not store diluted reagents**.

Storage/Stability
The Duolink PLA Multicolor Reagent Pack is shipped on dry ice. However, the 1× Blocking Solution and 1× 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 PLA 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 PLA Multicolor experiments.

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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