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# **Product Information**

#### Duolink® PLA Multicolor Probemaker Kit - Red

Product Number **DUO96010-1KT** Storage Temperature –20 °C

### **Product Description**

Duolink® PLA Multicolor Probemaker Kit - Red contains all the necessary reagents to perform the conjugations of Red Oligo A and Red Oligo B directly to a pair of primary antibodies. The resulting PLA probes (Red A and Red B) **must be used together and cannot be paired with any other Duolink PLA probe**. Following rolling-circle amplification, corresponding detection oligonucleotides with a fluorophore ( $\lambda_{ex} = 594$  nm and  $\lambda_{em} = 624$  nm) will allow detection when using the same filter as for Texas Red®.

Duolink PLA Multicolor Kits allow simultaneous detection of up to 4 protein events (protein interactions, post-translational modifications, and/or sensitive protein detection) within a fixed cell or tissue sample.

To perform a Duolink PLA Multicolor experiment, one will need pairs of primary antibodies (IF- or IHC-validated) that specifically recognize two target epitopes per Duolink PLA Multicolor Probemaker Kit. At least two pair of the resulting Duolink PLA Multicolor Probemaker-generated probes (Red A/Red B, Green C/Green D, Orange F/Orange G, and/or FarRed H/FarRed I) are needed. The reagents in the Duolink PLA Multicolor Reagent Pack are required to perform the amplification and detection of bound Multicolor PLA probes. Recommended additional reagents include Duolink Wash Buffers and Mounting Medium.

#### Components

Sufficient components are provided to independently conjugate 20  $\mu g$  of two antibodies at a concentration of 1 mg/mL.

**Red Oligo A** – Lyophilized, activated Red Oligo A oligonucleotide for one conjugation of 20 μg antibody. DUO86010A 1 vial

Red Oligo B – Lyophilized, activated Red Oligo B oligonucleotide for one conjugation of 20 μg antibody. DUO86010B 1 vial

Conjugation Buffer – For the conjugation reaction. DUO82033 55  $\mu$ L

**Stop Reagent** – For stopping the conjugation reaction. DUO82034 55  $\mu$ L

Storage Solution – For preserving the prepared PLA probes Red A and Red B (conjugated antibodies) DUO82035 2 vials of 60  $\mu$ L

## **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**

The Duolink PLA Multicolor Red Oligo A, Red Oligo B, Conjugation Buffer, Stop Reagent, and Storage Solution are supplied ready to use in the conjugation procedure. Vortex all liquid components before use.

The primary antibodies should have a concentration of 1 mg/mL.  $20~\mu g$  ( $20~\mu L$ ) of antibody is required per conjugation. The antibodies must be in an amine-free buffer, ideally PBS. The buffer should be carrier- and preservative-free, but may contain up to 0.1% BSA, 5% trehalose, and 0.02% sodium azide.

# Storage/Stability

Store the kit components at -20 °C.

Store the prepared PLA probes Red A and Red B (conjugated antibodies) at 2–8 °C. **Do not freeze.** 

Note: The Storage Solution contains buffer and reagents for stabilizing the conjugated antibodies. Other components may need to be added to preserve specific antibodies.

#### **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.

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