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Product Information

Duolink® In Situ Detection Reagents Brightfield

Catalog Number **DUO92012**

Product Description

Duolink® In Situ Detection Reagents Brightfield contains all the necessary reagents to perform the amplification and detection of bound PLA® probes. The read-out is performed by brightfield microscopy.

Experiments conducted using Duolink In Situ reagents can detect and visualize protein interactions, protein expression levels, and post translational modifications at the single molecule level in fixed cells and tissue samples.

To perform a complete Duolink In Situ experiment, one will need two primary antibodies (IHC or ICC/IF validated) that recognize two target epitopes. Additional reagents required include a pair of PLA probes (one PLUS and one MINUS) and detection reagents of choice. Recommended reagents include Wash Buffers and Mounting Medium.

Components

Box A, Catalog Number DUO92012A (30 or 100 RXN)
Sufficient components are provided in Box A for the indicated number of reactions (30 or 100 RXN), based on 40 μL of the total reaction mixture covering 1 cm^2.

5× Ligation – Contains oligonucleotides that hybridize to the PLA probes and all components needed for ligation except the Ligase.
30 RXN – Catalog Number DUO82009
100 RXN – Catalog Number DUO82009

1× Ligase (1 unit/μL)
30 RXN – Catalog Number DUO82029
100 RXN – Catalog Number DUO82027

5× Amplification – Contains all components needed for Rolling Circle Amplification (RCA) except the Polymerase.
30 RXN – Catalog Number DUO82050
100 RXN – Catalog Number DUO82050

5× Detection Brightfield – Contains oligonucleotide probes labeled with horseradish peroxidase (HRP) that hybridize to the RCA product.
30 RXN – Catalog Number DUO82051
100 RXN – Catalog Number DUO82051

1× Polymerase (10 units/μL)
30 RXN – Catalog Number DUO82030
100 RXN – Catalog Number DUO82028

Box B, Catalog Number DUO92012B
Sufficient components are provided in Box B for either 30 or 100 RXN, based on 40 μL of the total reaction mixture covering 1 cm^2.

1× Hydrogen Peroxide – Contains 0.3% hydrogen peroxide, which is used to quench endogenous peroxidase activity.
Catalog Number DUO82054

Substrate Reagents A–D – Contains all substrate components needed for HRP enzymatic reaction.

Substrate Reagent A, Catalog Number DUO82055
Substrate Reagent B, Catalog Number DUO82056
Substrate Reagent C, Catalog Number DUO82057
Substrate Reagent H_2O_2 (D),
Catalog Number DUO82058

1× Nuclear Stain – Contains Mayer's hematoxylin solution for cell nuclei staining.
Catalog Number DUO82059

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× Ligation, 5× Amplification, and 5× Detection Brightfield components at room temperature and vortex before use. Dilute the required volumes of each 5× solution 5-fold with high purity water **immediately before use**. **Do not store diluted reagents**.
Note: The 5× Ligation component contains DTT that may precipitate at –20 °C. The DTT doesn't need to be completely dissolved, but ensure the tube is thoroughly vortexed before use.

The Ligase and Polymerase enzyme solutions 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.

The 1× Hydrogen Peroxide and 1× Nuclear Stain are supplied ready to use.

Substrate Reagents A–D – Thoroughly vortex and quick spin each reagent before pipetting. Prepare 1× working substrate solution by diluting the Substrate Reagents A–D in high purity water in the following ratios: Substrate A, 1:70; Substrate B, 1:100; Substrate C, 1:100; and Substrate H_2O_2 (D), 1:50. Vortex after addition of each reagent. Optimal staining intensity is generally observed with a 5–10 minute incubation; however, longer incubation may increase sensitivity.

Do not store diluted reagents.

Storage/Stability
Store Box A and all of its 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.

Store Box B and all of its components at 2–8 °C.

Procedure
The experimental procedures for Duolink In Situ Brightfield applications can be found at sigma.com/duolink.

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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