

Product Information

Duolink® In Situ Detection Reagents Orange

Catalog Number **DUO92007**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

Product Description

Duolink® In Situ Detection Reagents Orange contains all the necessary Duolink In Situ reagents to perform the amplification and detection of bound PLA® probes. The detection probes contain a fluorophore ($\lambda_{\text{ex}} = 554\text{ nm}$ and $\lambda_{\text{em}} = 576\text{ nm}$), which may be visualized using the same filter as Cy3™.

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

Sufficient components are provided for the indicated number of reactions (30 or 100 RXN), based on $40\text{ }\mu\text{L}$ 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

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

5× Amplification Orange – Contains all components needed for Rolling Circle Amplification (RCA) except the Polymerase. It also contains oligonucleotide probes labeled with a fluorophore that hybridize to the RCA product.
30 RXN – Catalog Number DUO82010
100 RXN – Catalog Number DUO82010

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 and 5× Amplification Orange 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 $-20\text{ }^{\circ}\text{C}$. 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 ($-20\text{ }^{\circ}\text{C}$) 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 components at $-20\text{ }^{\circ}\text{C}$. The enzymes should be kept cold ($-20\text{ }^{\circ}\text{C}$) at all times, use a freezing block when removing them from the freezer.

Procedure

The experimental procedures for Duolink In Situ fluorescence 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.

Duolink and PLA are registered trademarks of Sigma-Aldrich Co., LLC.
Cy3 is a trademark of GE Healthcare.

PCG,ANA,MAM 04/17-1