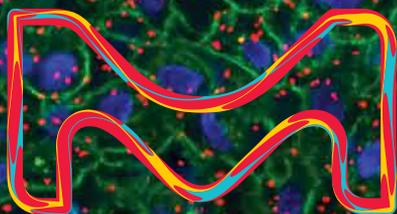


MILLIPORE
SIGMA

Amplified Detection

Duolink® Proximity Ligation Assay (PLA)



The life science
business of Merck
KGaA, Darmstadt,
Germany operates as
MilliporeSigma in the
U.S. and Canada.

Sigma-Aldrich®
Lab & Production Materials

Inside every cell, there is a breakthrough waiting to be uncovered. The chance to change the origin of a human disease or discover the tell-tale signal of an irregular protein-protein interaction requires powerful investigative tools and a streamlined approach. Rely on Duolink® PLA to amplify protein signals by 1000x, allowing you to visually detect proteins and their interactions, all with a straightforward protocol.

Uncover the Opportunities in Your Research with Amplified Performance and Visual Results

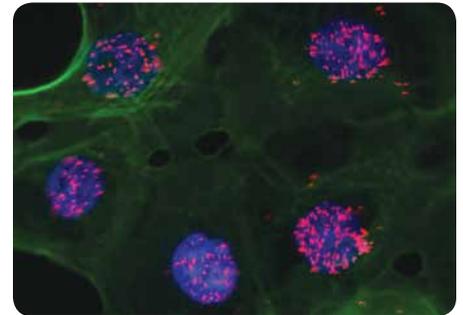
- **Visualize protein interactions** – both stable and transient
- **Detect endogenous proteins** – no overexpression or genetic manipulation
- **High specificity** – use of two antibodies/probes eliminates false positives
- **Single molecule sensitivity** – rolling circle amplification makes proteins visible
- **No special equipment needed** – standard immunofluorescence methods
- **Publication-ready results** – compare image results from indirect methods (Co-IP/WB)

Applications of Duolink® PLA

Protein-Protein Interaction Detection and Quantification

Proteins influence cellular function by interacting with other proteins, DNA, membrane components, structural components, etc. Duolink® PLA allows you to see these interactions within the cell, with clear, visual signals. The signal is generated only if the proteins of interest are within 40nm, therefore detecting interaction.

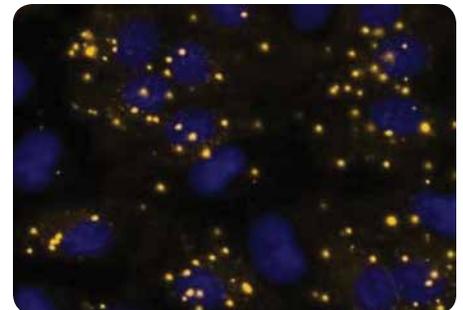
Image shows detection of EZH2-H3K27me3 interaction with Duolink® PLA.



Post-Translational Modification (PTM) Detection and Quantification

Reliably detecting protein modifications, such as methylation or phosphorylation, can be challenging. Duolink® PLA allows you to detect PTMs with specificity and sensitivity. Use two antibodies, one for the protein, and one for its modification of interest. If the modification is present on the protein, then both antibodies should bind and generate a signal.

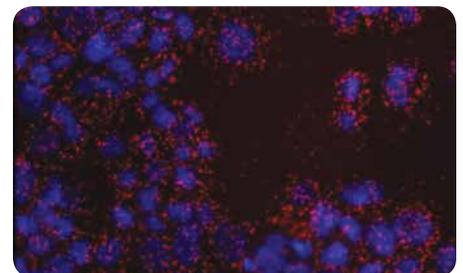
Image shows detection of pEGFR with Duolink® PLA.



Low Expression Protein Detection and Quantification

Low abundance proteins and biomarkers can be missed due to lack of detection sensitivity with traditional techniques. Duolink® PLA allows you to detect as few as a single event. As long as you have the target specific antibodies, you will detect the protein. No overexpression or genetic manipulation is needed.

Image shows detection of EGFR with Duolink® PLA.

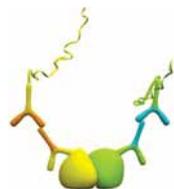


Next-Level Immunodetection Technology

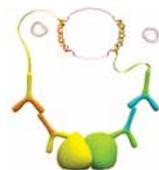
Duolink® PLA is based on the proximity ligation assay (PLA) principle, and combines the specificity of secondary antibodies with the sensitivity afforded by rolling circle amplification to detect endogenous proteins in fixed cells and tissues. A pair of oligonucleotide labeled antibodies (PLA probes) generates an amplified signal only when the probes are in close proximity (<40nm).



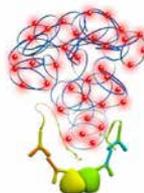
Two antibodies raised in separate species bind two proteins of interest or two epitopes of a single protein.



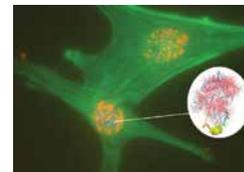
Secondary antibodies with PLA probes (PLUS and MINUS) are added



Two circle-forming DNA oligonucleotides and the ligation enzyme are added



Polymerase amplifies the DNA circle. Fluorescently labeled, complementary oligonucleotide probes bind to the amplified DNA.



Each signal is composed of ~1000 bound fluorescent probes that appear as a distinct dot that can be easily visualized under a microscope.

Simple Protocol

With Duolink® PLA, you can detect, quantify, and visualize protein-protein interactions, post-translational modifications, and low expression protein detection with an easy-to-execute immunodetection protocol that is similar to traditional IF or IHC experiments, but yields far more sensitive signals.

Further your protein discovery with this simple and straightforward protocol that involves eight basic steps:

1. **SAMPLE PREPARATION** (cell or tissue slides, fixed and permeabilized)
2. **BLOCKING**
3. **PRIMARY ANTIBODY INCUBATION**
4. **PLA PROBE ADDITION**
5. **LIGATION** (hybridization of the connector oligos to PLA probe arms and ligation to form a template for rolling circle amplification (RCA))
6. **AMPLIFICATION** (and labeling of the RCA product by detection probes)
7. **SLIDE PREPARATION** (washes and coverslip mounting)
8. **IMAGING AND ANALYSIS** (use a fluorescent microscope)

For additional guidance on establishing protocols and executing experiments contact our experienced technical support team at techserv@sial.com.

Duolink® PLA Products

To perform a Duolink® PLA experiment, you will need the following:

- Cells or tissue samples (prepared for PLA experiments - mounted, fixed, permeabilized)
- Two primary antibodies (IHC, ICC or IF validated) generated in different hosts (mouse, rabbit or goat)
- Two PLA probes, one PLUS and one MINUS to detect the primary antibodies
- Detection reagents, fluorescent color of choice or brightfield
- Wash buffers
- Mounting medium

Recommended accessories include:

- hydrophobic pen
- humidity chamber
- heat transfer block
- freezer block
- staining jar
- shaker

Duolink® PLA Starter Kits

The Starter Kit contains all the necessary reagents you need to perform a Duolink® PLA experiment and analyze up to 30 samples. All you have to provide are prepared cells or tissue samples, primary antibodies, and common laboratory equipment.



Select one of the six kits below based on the primary antibody species and desired detection color:

Product No.	Description
DUO92101	Duolink® <i>In Situ</i> Red Starter Kit Mouse/Rabbit
DUO92102	Duolink® <i>In Situ</i> Orange Starter Kit Mouse/Rabbit
DUO92103	Duolink® <i>In Situ</i> Red Starter Kit Mouse/Goat
DUO92104	Duolink® <i>In Situ</i> Orange Starter Kit Mouse/Goat
DUO92105	Duolink® <i>In Situ</i> Red Starter Kit Goat/Rabbit
DUO92106	Duolink® <i>In Situ</i> Orange Starter Kit Goat/Rabbit

Once you become familiar with Duolink® PLA, you can choose to customize your experiments and select specific reagents to fit your need.

How Duolink® PLA Technology Compares

Capabilities	Duolink®	IP/Western	FRET
Interaction Type	Stable, transient or weak interactions	Primarily high-affinity interactions	Stable interactions
Localization	✓	✓	✓
Quantification	✓ Software specific	✗ Semi-quantitative	✓
Endogenous Protein	✓	✗ Overexpression is often required	✗ Requires genetic modification of two proteins
Sensitivity	✓✓✓ Very high, single molecule detection	✗ Low sensitivity, low accuracy	✗ Moderate
Cells and Tissue	✓✓ Cells and tissue (frozen or FFPE)	✓ Cell and tissue lysates	✓ Live cells

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The Duolink® PLA Control Kit – PPI

The Duolink® PLA Control Kit – PPI (DUO92202) applies the well-established protein-protein interaction of EGFR and HER2 to provide complete confidence in interpreting your results for a more efficient workflow. The optimal primary antibody concentrations have already been determined and recommendations for conditions are provided.

Each kit contains:

- Two 8-well chamber slides with EGF-treated pre-fixed SKOV3 cells
- Mouse anti-EGFR
- Rabbit anti-HER2 primary antibodies

To execute the Duolink® assay, you will need one of the following starter kits:

- Duolink® *In Situ* Red Starter Kit Mouse/Rabbit (DUO92101)
- Duolink® *In Situ* Orange Starter Kit Mouse/Rabbit (DUO92102)

Duolink® PLA Resource Center

Visit us online to learn more about Duolink® PLA technology and how it can amplify your research. Our Resource Center provides the tools you need to become well-versed in protein detection technology, including:

- Application notes
- Protocols and guides
- Instructional videos
- On-demand webinars
- Publications and more

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