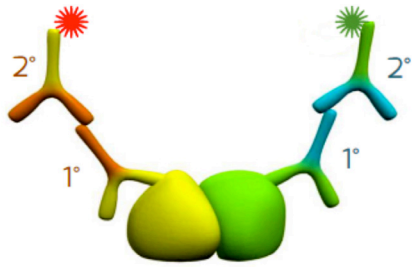
Sigma-Aldrich®
Lab Materials & Supplies

An Introduction to Duolink® PLA

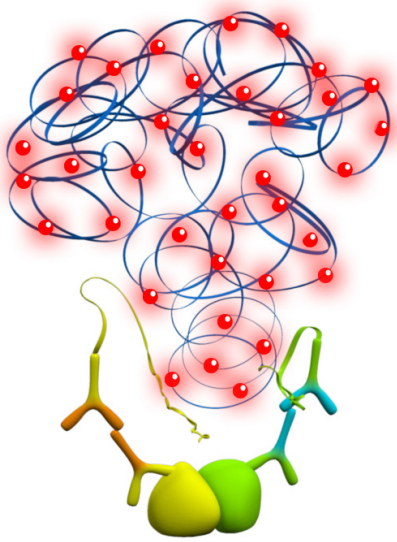
Protein Detection Technology

Duolink® PLA protein detection technology is based on *in situ* proximity ligation assay (PLA), and allows for the detection, visualization, and quantification of protein expression, protein-protein interactions, and post-translational modifications, in unmodified cells and tissue samples within a matter of hours.

Duolink® PLA utilizes a pair of oligonucleotide-labeled secondary antibodies (PLA probes). Signal is generated only when the two PLA probes have bound in close proximity. Rolling-circle amplification (RCA) provides up to 1000-fold increase in the signal from each detected pair of PLA probes, visualized as an individual fluorescent spot. These PLA signals can be quantified and assigned to a specific subcellular location based on cellular images.



Immunodetection with
traditional Secondary Antibodies



Immunodetection with
Duolink® PLA

Features & Benefits

Duolink® PLA has several advantages over traditional immunodetection methods:

- **Visualize protein interactions** – stable, transient, or weak interactions
- **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** – visual data confirms proteins and interactions

MilliporeSigma

Assay Principle

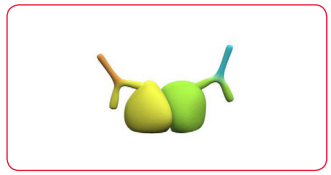
Duolink® PLA combines the specificity of secondary antibodies with the sensitivity afforded by rolling circle amplification (RCA) to detect endogenous proteins in fixed cells and tissues. The most common usage of Duolink® PLA technology is for the *in situ* detection of protein-protein interactions. Two primary antibodies (raised in different species) are used to detect two different protein targets. A pair of secondary antibodies conjugated with oligonucleotides (PLA PLUS and MINUS probes) bind specifically to the primary antibodies. The PLA probes will participate in RCA only when the PLUS and MINUS oligonucleotides are in close proximity (<40nm). This reaction results in up to 1000-fold amplification. Labeled complementary oligonucleotides allow detection by microscopy or flow cytometry.

Workflow Overview

The Duolink® PLA workflow is outlined below. Detection of protein-protein interaction is given as an example.

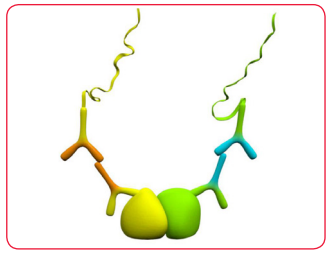
1. Primary Antibody Incubation

Fixed samples are incubated with two primary antibodies raised in separate species (mouse, rabbit, or goat) that bind to the proteins of interest.



2. PLA Probe Incubation

Secondary antibodies conjugated with oligonucleotides (PLA probe MINUS and PLA probe PLUS) are diluted, added to the samples, and incubated.

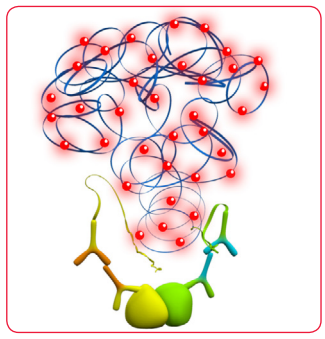


3. Ligation

The Ligation Solution, consisting of two bridging DNA oligonucleotides (illustrated as red bands) and a ligation enzyme (ligase), is applied. The DNA oligonucleotides hybridize to the two PLA probes and the enzyme will form a ligated (closed) circle, if the probes are in close proximity (<40nm apart).

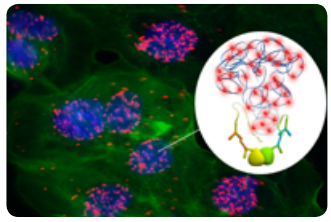
4. Amplification

The Amplification Solution, consisting of nucleotides, fluorescently labeled, complementary oligonucleotides, and a polymerase, is applied. One of the PLA probes acts as a primer for the polymerase, generating a repeating, concatemeric product which is still tethered to the secondary antibody. Labeled oligonucleotides then hybridize to the amplified DNA to yield a signal that is easily detectable by fluorescence or brightfield microscopy.



5. Imaging and Analysis

Data analysis is the final step. Each PLA signal is composed of ~1000 bound fluorescent probes that together appear as a distinct dot that can be easily visualized under a microscope or detected by flow cytometry. Imaging software can be used to obtain quantification of the PLA signals and subcellular localization (nuclear or cytoplasm) for microscopy experiments, enabling single cell statistical analysis in a tissue or cell population.



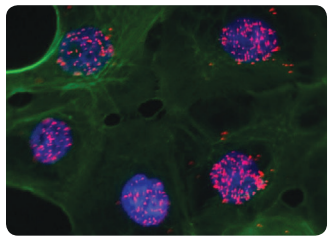
Applications of Duolink® PLA

Duolink® PLA allows easy detection and quantification of protein expression, protein-protein interactions, and post-translational modifications in unmodified cells and tissue samples. Duolink® PLA can also be used to detect protein-DNA and protein-RNA interactions when used in combination with modified in situ hybridization (ISH) techniques. Whatever the application, using quality, specific antibodies to your target(s) of interest will allow Duolink® PLA to help you detect them.

Detection of Protein-Protein Interactions (PPI)

The human genome project predicted 130,000 - 600,000 PPIs. Since many biologic targets of disease involve PPIs, mapping the protein interactome is of great importance. Duolink® PLA allows you to detect these interactions within the cell, with clear visual signals. The signal is generated only if the two proteins of interest are within 40nm, strongly suggesting an interaction.

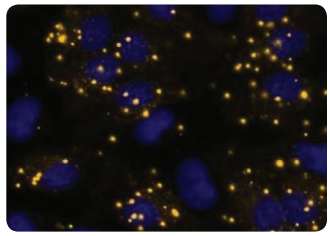
Image shows detection of EZH2-H3K27me3 interaction in DU145 cells using Duolink® PLA



Detection of Post-translational Modifications (PTM)

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

Image shows Duolink® PLA detection of pEGFR following EGF stimulation of SKOV-3 cells



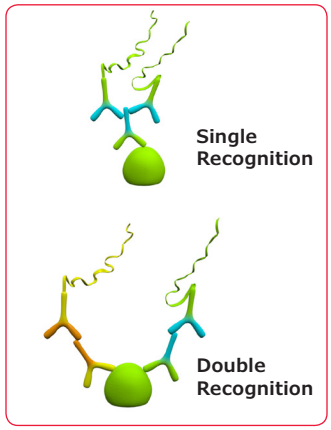
Detection of Protein Expression

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

There are two ways to detect a single protein target: with the use of one primary antibody (single recognition) or two primary antibodies (double recognition) against the target.

Single recognition confers the highest sensitivity and thus is recommended when detecting single protein targets present at low expression levels.

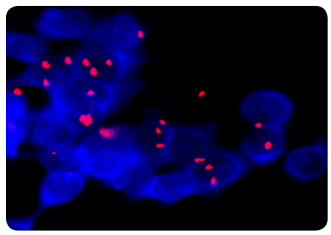
Double recognition allows single protein detection with greater specificity through the use of two primary antibodies directed against two different epitopes on the same protein.



Other Uses of Duolink® PLA

Duolink® PLA is a highly sensitive, antibody-based detection technology. Its versatility can be exploited for the detection of protein-DNA interactions, protein-RNA interactions, and even gene-specific modifications (no proteins!). These events can be detected through a combination of modified in situ hybridization and Duolink® PLA techniques.

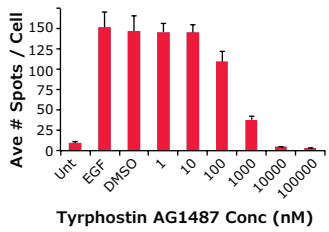
Image shows Duolink® PLA detection of Septin 9 promoter methylation in SW480 cells (triploid on chromosome 17)



Relative Quantitation

Duolink® PLA results can be quantified either by counting the number of PLA signals (spots) per cell or subcellular localization by microscopy or by signal intensity per cell by flow cytometry. This allows single cell statistical analysis in a tissue or cell population.

Figure shows the dose effects of a Tyr kinase inhibitor on EGF-induced phosphorylation of EGFR in SKOV-3 cells detected by Duolink® PLA



Detection Formats

Duolink® PLA products are available for fluorescence and brightfield. Fluorescence Duolink® PLA reagents can currently be used in both microscopy and flow cytometry application formats.

Fluorescence Detection

Fluorescent microscopy is the most commonly used application format for Duolink® PLA experiments. Much of the protocol is the same when using Fluorescence Duolink® PLA reagents as traditional immunofluorescence (IF), however, Duolink® PLA exhibits greater specificity and versatility than IF. The added amplification step included in the Duolink® PLA procedure provides much greater sensitivity.

Brightfield Detection

As with traditional immunohistochemistry (IHC), Brightfield Duolink® PLA is predominantly used on formalin-fixed, paraffin-embedded tissue sections, but is compatible with other sample types. Brightfield Duolink® PLA detection reagent contains oligonucleotides labeled with horseradish peroxidase (HRP) that, in the presence of an HRP substrate, forms an enzyme:substrate precipitate visible using a light microscope.

Flow Cytometry

Flow cytometry offers several advantages over fluorescent microscopy, including the rate at which a sample can be analyzed and more powerful statistical analysis. However, there are limitations to flow cytometry as well, such as identifying protein-protein interactions or subcellular localization. Combining Duolink® PLA with flow cytometry will allow collection of quantitative data in a high throughput manner.

For more information, visit
SigmaAldrich.com/duolinkpla

Duolink® PLA Starter Kits

Getting started with Duolink® PLA is easy. The protocols are very similar to that of IF, IHC, and flow cytometry, though Duolink® PLA offers distinct advantages over these and other traditional methods of protein detection.

Duolink® PLA Starter Kit

The Duolink® PLA 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. A Duolink® PLA Starter Kit includes:

- Two PLA probes: one PLUS and one MINUS (match to the host of the primary antibodies)
- Antibody diluent and blocking buffer
- Detection reagent: choice of red or orange
- Amplification buffer and Polymerase enzyme
- Ligation Stock and Ligase enzyme
- Wash buffers A and B
- Mounting medium with DAPI

Resources

In addition to the Duolink® PLA Protocols for Fluorescence, Brightfield, and Flow Cytometry, be sure to visit our online resource center for details on how to successfully set up and execute a Duolink® PLA experiment.

- How to Optimize the Duolink® Proximity Ligation Assay (PLA)
- Product Selection Guide
- Troubleshooting & FAQ
- Instructional videos and more

To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**
Technical Service: **SigmaAldrich.com/techservice**
Safety-related Information: **SigmaAldrich.com/safetycenter**

SigmaAldrich.com

Lit. No. 4383_MSIG Ver. 1.0
2017 - 04383
05/2017