Amplified Detection

Duolink® Proximity Ligation Assay (PLA)
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).

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
Publication-Ready Results

Duolink® PLA offers several key advantages over traditional methods that make it the next-level of immunodetection technology. These include:

- Detection of proteins at endogenous levels without overexpression or genetic manipulation
- Visual detection of protein interactions within the cell
- Amplified signal that makes even single events visible

Publish more compelling data with Duolink® PLA.

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:

<table>
<thead>
<tr>
<th>Product No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUO92101</td>
<td>Duolink® In Situ Red Starter Kit Mouse/Rabbit</td>
</tr>
<tr>
<td>DUO92102</td>
<td>Duolink® In Situ Orange Starter Kit Mouse/Rabbit</td>
</tr>
<tr>
<td>DUO92103</td>
<td>Duolink® In Situ Red Starter Kit Mouse/Goat</td>
</tr>
<tr>
<td>DUO92104</td>
<td>Duolink® In Situ Orange Starter Kit Mouse/Goat</td>
</tr>
<tr>
<td>DUO92105</td>
<td>Duolink® In Situ Red Starter Kit Goat/Rabbit</td>
</tr>
<tr>
<td>DUO92106</td>
<td>Duolink® In Situ Orange Starter Kit Goat/Rabbit</td>
</tr>
</tbody>
</table>

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

How does Duolink® PLA Compare?

<table>
<thead>
<tr>
<th>Capabilities</th>
<th>Duolink</th>
<th>IP/Western</th>
<th>FRET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interaction Type</td>
<td>Stable, transient or weak interactions</td>
<td>Primarily high-affinity interactions</td>
<td>Stable interactions</td>
</tr>
<tr>
<td>Localization</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Quantification</td>
<td>✓</td>
<td>x</td>
<td>✓</td>
</tr>
<tr>
<td>Endogenous Protein</td>
<td>✓ x</td>
<td>Overexpression is often required</td>
<td>Requires genetic modification of two proteins</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>✓ ✓ ✓ Very high, single molecule detection</td>
<td>✓ Low sensitivity, low accuracy</td>
<td>✓ Moderate</td>
</tr>
<tr>
<td>Cells and Tissue</td>
<td>✓ ✓ Cells and tissue (frozen or FFPE)</td>
<td>✓ Cell and tissue lysates</td>
<td>✓ Live cells</td>
</tr>
</tbody>
</table>

Duolink® PLA Resource Center

Visit us online to learn more about Duolink® PLA 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

SigmaAldrich.com/duolink