

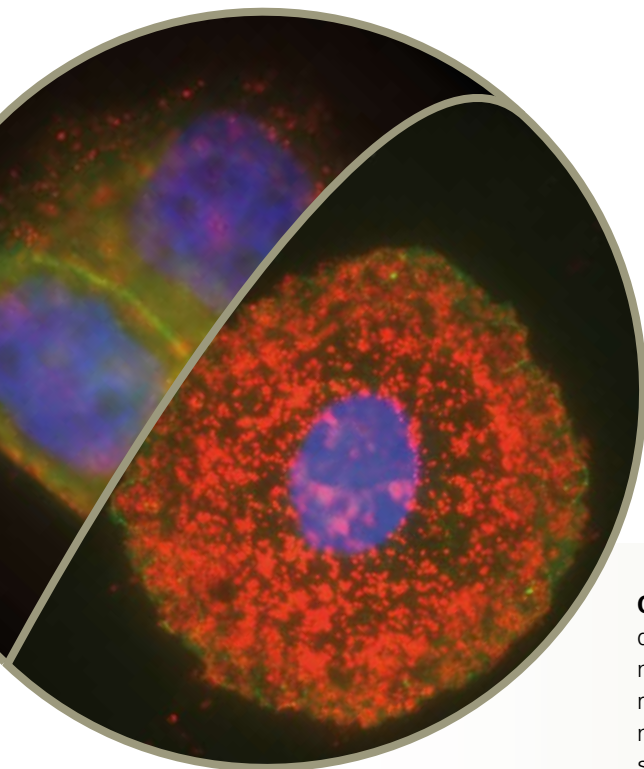
A Revolution in the Detection of Endogenous Proteins?

With Duolink Proximity Ligation Assay *in situ* (PLA *in situ*) reagent kit you can:

- Detect proteins, protein interactions and protein modifications.
- Visualize single molecular events.
- Digitally count detected events.
- Analyze protein targets in cells and tissues at physiological levels.
- Determine the location of protein targets.

PLA *in situ* combines highly specific target recognition (due to the requirement for double recognition) with powerful signal amplification (due to DNA amplification) for straightforward detection of single protein events in unmodified cells and tissues.

Duolink detects endogenous protein interactions, e.g. ligand binding of membrane proteins and intracellular proteins, through a pair of antibodies that binds to proteins in close proximity. Individual protein interactions are made visible through DNA amplification. The level of activity can be accurately and objectively quantified by counting fluorescent dots in the cell.



With Duolink™, you get a clear signal from individually detected proteins or protein complexes, enabling you to quantitatively visualize endogenous proteins in signaling pathways, *in situ* in individual cells. The images show the presence of HER2 dimerization at the cell surface of MDA-231 cells (left) and HER2-positive SKBR-3 cells (right).

Olink Biosciences is a privately held company based in Uppsala, Sweden, founded in 2004 by Prof. Ulf Landegren and partners. Olink focuses on innovative methods for detecting endogenous proteins and protein complexes for basic research and high-content screening to deliver accurate information on disease mechanisms and drug response. Our mission is to contribute to a better understanding of the interactome. For more information, visit www.olink.com.

Principle of Duolink assay

Fig 1:

A pair of primary antibodies (brown and red), one of mouse origin and one of rabbit origin, binds to the protein(s) to be detected. The two PLA probes (grey) are secondary antibodies, one anti-mouse Ig and one anti-rabbit Ig, conjugated with oligonucleotides. These are added and they bind to their respective primary antibody.

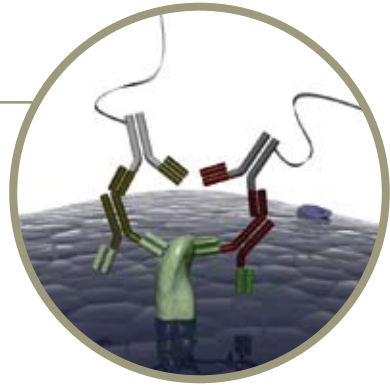


Fig 2:

Two oligonucleotides (red bands) are added together with a ligase (not shown), forming a circle when the PLA probes are in close proximity.

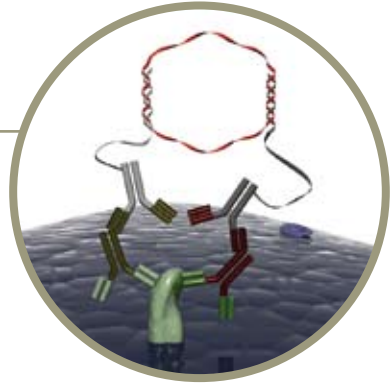


Fig 3:

Polymerase (yellow) and nucleotides (not shown) are added. The oligonucleotide arm of one of the PLA probes acts as a primer for a rolling circle amplification (RCA) reaction using the ligated circular oligonucleotide as a template, generating a concatemeric (repeated sequence) product extending from the oligonucleotide arm of one of the PLA probes.

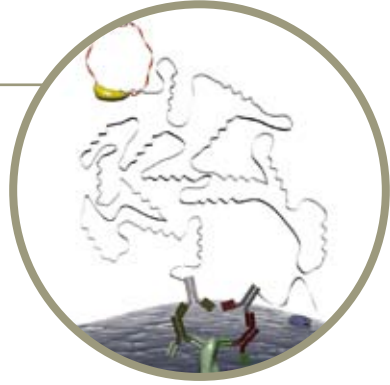
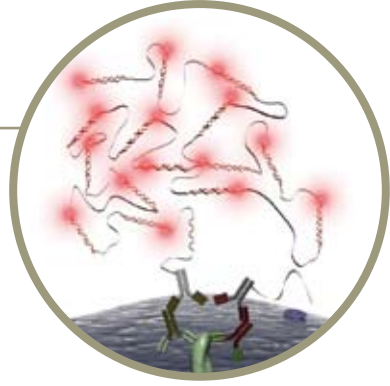


Fig 4:

Fluorescently labeled probes, complementary in sequence to the RCA product, hybridize to the RCA product. The signal is easily visible by fluorescence microscopy.



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