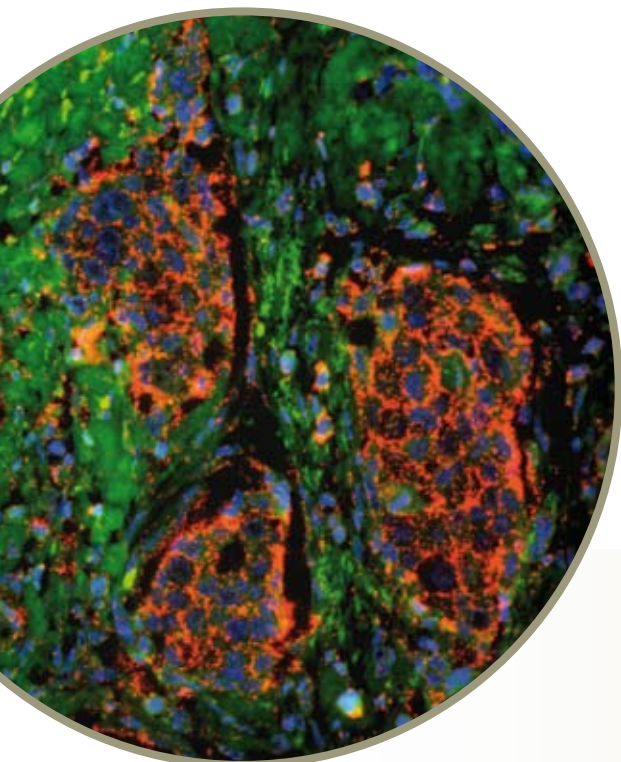


Characteristics of Proximity Ligation Assay *in situ* compared to other in-cell detection methods

- Enables the study of protein interactions in unmodified cell lines and primary cultures without altering expression levels.
- Makes feasible the study of protein interactions in normal and neoplastic material, visualizing the interaction *in situ*.
- Can be directly applied on collected tissue bank materials, both fresh frozen and paraffin embedded samples.
- Exhibits profoundly enhanced specificity compared to assays that depend on single binding events.
- Determines the location of the target protein because each signal is a discrete detection of a single event.
- Only requires standard lab equipment.

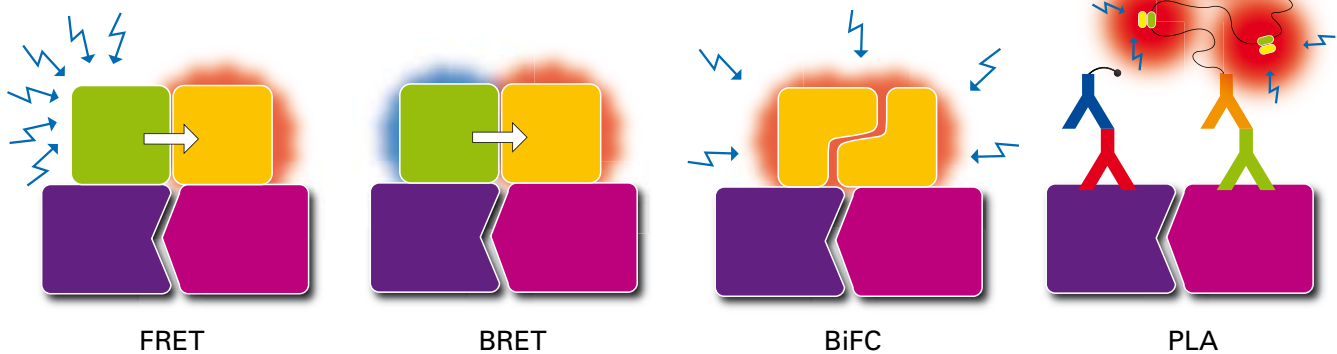
Duolink™ is a unique approach for detecting interactions between normal proteins at physiological levels of expression. It is ideal for studies of endogenous protein interactions and signaling pathways in cell lines as well as tissue biopsies.



Detection of endogenous HER2 in tissue banked formalin fixed paraffin embedded breast cancer tumor using Duolink. Red: HER2; green: autofluorescence; blue: Hoechst-stained nuclei. 20X/0.8, epifluorescence.

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.

There is a great need to better understand disease pathways. To localize the relevant proteins and their interactions in morphologically conserved unmodified cells, more refined yet simple methods are required. Proximity Ligation Assay *in situ* (PLA *in situ*) provides you with that tool. It is a highly selective, sensitive and simple method enabling quantitative visualization of single protein molecular events in individual cells, with important advantages over existing methods.



Methods that allow in-cell visualization of protein complexes include Fluorescent Resonance Energy Transfer (FRET), Bioluminescence Resonance Energy Transfer (BRET), Biomolecular Fluorescence Complementation (BiFC) and Proximity Ligation Assay (PLA). PLA is the only method that can be used on unmodified cells and tissues, whereas FRET, BRET and BiFC act on live, genetically modified cells with usually overexpressed fusion protein complexes. Only in the case of PLA are endogenous, single protein complexes detected with an amplifiable signal, thereby enabling the detection of single protein events. *Adapted from Nature Methods, Dec 2006.*

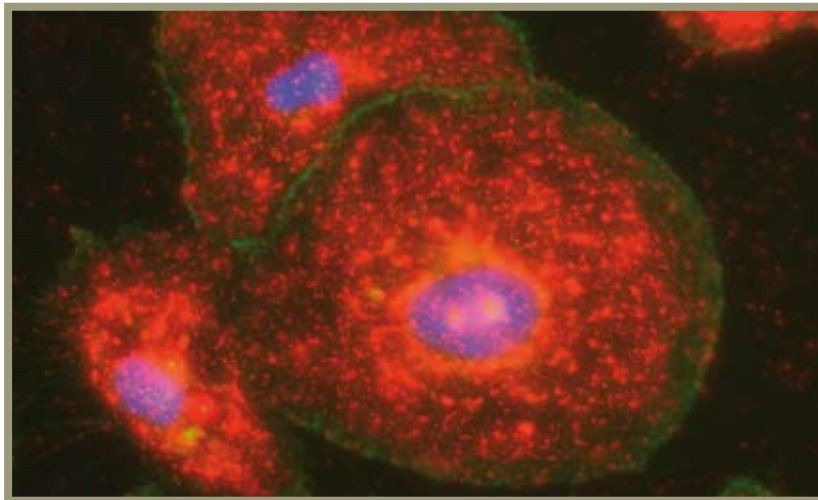
Specificity and sensitivity for single protein events, with a simple method

PLA *in situ* exhibits profoundly enhanced specificity over assays that depend on single binding recognition. This is because PLA *in situ* requires positive identification of two different epitopes on the same protein or complex. This, combined with signal amplification generated by an amplifiable DNA strand that serves as a target for multiple fluorescent molecules, enables reliable and straightforward on-site visualization of a single protein event with standard laboratory equipment and a standard fluorescence microscope.

Comparison of current methods for in-cell visualization of protein complexes

Established methods to detect proteins *in situ* include FRET, BRET, and BiFC. The table below summarizes some of the important characteristics of these methods in comparison with PLA *in situ*.

	Protein identification	Localized detection	Endogenous proteins	Protein interactions	Analyzing living cells	Analyzing tissue bank	Unmodified cells
FRET		✓		✓	✓		
BRET		✓		✓	✓		
BiFC		✓		✓	✓		
PLA	✓	✓	✓	✓		✓	✓



Detection of endogenous HER2 dimers in the breast cancer cell line SKBR3 with Duolink. Red: HER2 dimers; blue: Hoechst-stained nuclei; green: Actin. 40X/1.3, epifluorescence, maximum intensity projection.

Examples of analytes

The following interactions have been successfully detected using PLA *in situ*.

Myc – Max
Myc-Max – RNA Pol
PDGFRB phosphorylation
HER2 – EGFR
HER2 – HER2
Max – Mad
VEGFR-2 phosphorylation
SMAD3 – SMAD4
IKK α – IKK β
HER2 – HER3

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