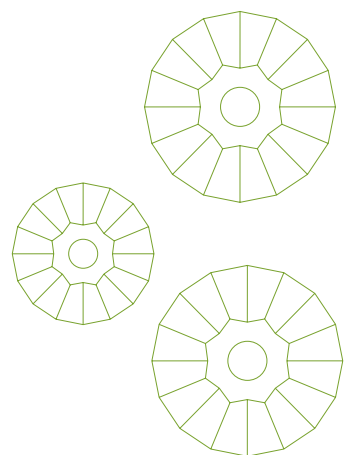
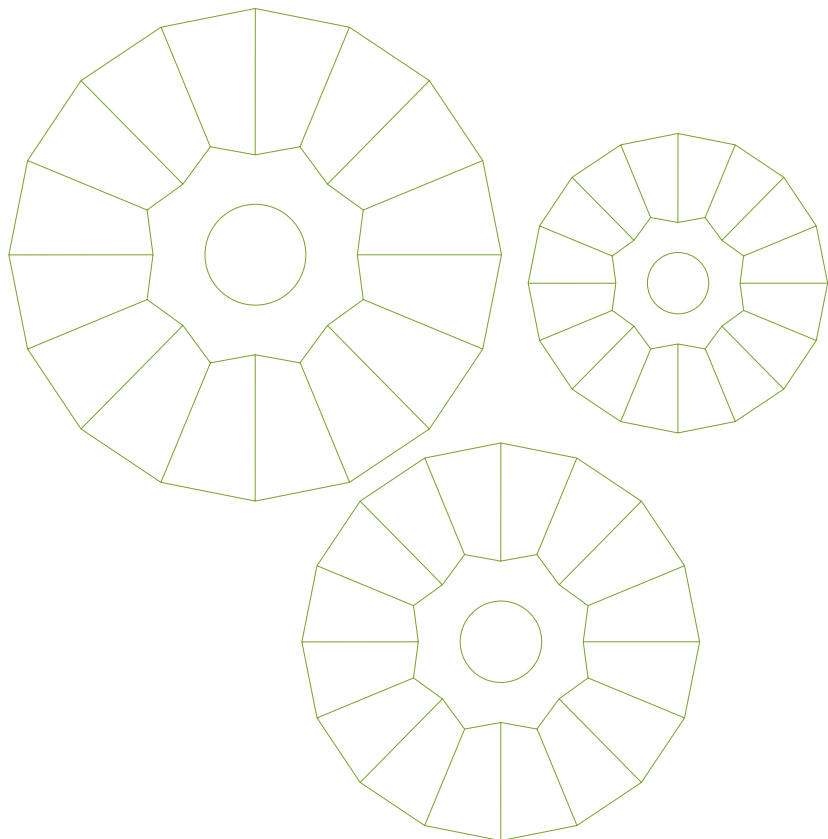


# Duolink<sup>®</sup>

Discover more about proteins and protein interactions





# Introduction

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The Duolink® approach provides new insights into proteins and protein interactions. For the first time, individual proteins, their interactions and modifications, can be accurately and objectively quantified in unmodified cells and tissues. Utilizing only a few cells, sub-cellular events, even transient or weak interactions, are revealed *in situ* and sub-populations of cells can be differentiated. Within hours, results from conventional co-immunoprecipitation and co-localization techniques can be confirmed.

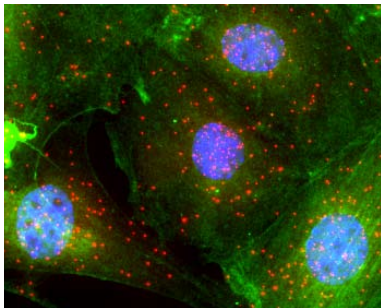
# Visualize and locate protein interactions

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The Duolink kits are based on *in situ* PLA<sup>®</sup>, a technology that extends the capabilities of traditional immunoassays to include direct detection of protein interactions with unparalleled specificity and sensitivity. For the first time, any target can be readily detected and localized with single molecule resolution. The following pages illustrate the advantages of utilizing this approach.

## 1. SIMULTANEOUSLY VISUALIZE AND LOCATE PROTEIN-PROTEIN INTERACTIONS

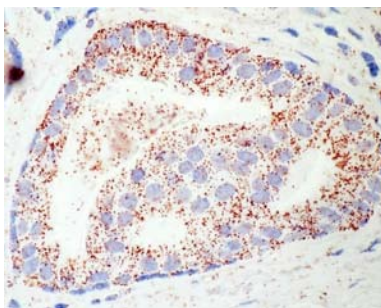
A Duolink kit reveals individual proteins and their interactions as quantifiable fluorescent signals or chromogenic stains. Each kit can be configured to meet specific assay requirements.



**Fig 1.** Single event resolution. Each red spot represents an SMAD 1/2/3 and SMAD 4 interaction in mouse embryonic fibroblasts.  
Green: FITC-anti-actin.  
Blue: DAPI stained nuclei.

## 2. SEE SUB-CELLULAR EVENTS *IN SITU*

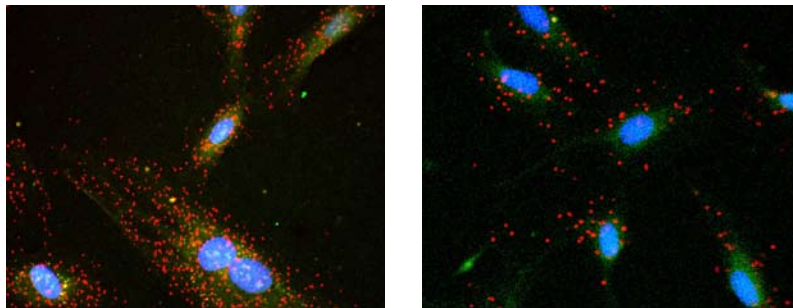
Retain protein localization. No modification of cells or tissue. No cell lysis, no over-expression, no interference from tags.



**Fig 2.** Breast cancer tissue (Duolink Detection Reagents Brightfield).  
Brown: HER2/HER3 interactions.

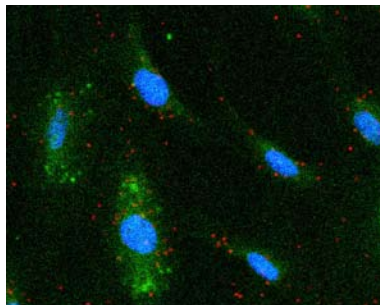
### 3. REVEAL TRANSIENT AND WEAK INTERACTIONS *IN SITU*

Detection and quantification of VE-PTP/VEGFR2 complexes *in situ* in endothelial cells treated or not with ligand (VEGF) for different time periods.

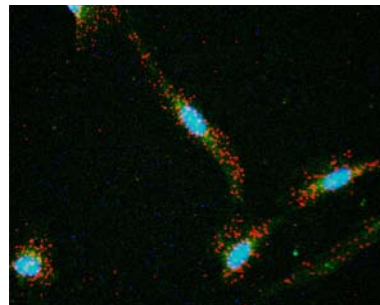


0 min – Unstimulated

5 min – Stimulated



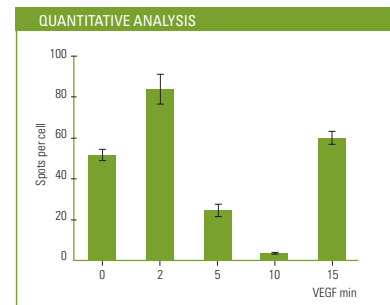
10 min – Stimulated



15 min – Stimulated

**Fig 3.** Red: VE-PTP/VEGFR2 complexes. Blue: Hoechst stained nuclei.

Reproduced through the courtesy of FASEB Journal. Ref: Mellberg et al. FASEB J. 2009 May;23(5):1490-1502.



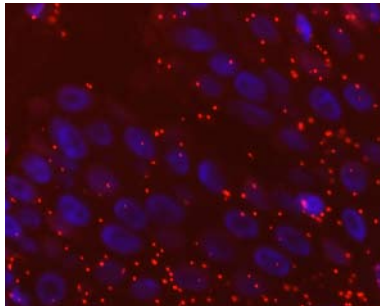
**Fig 4.** Quantification of VE-PTP/VEGFR2 complexes.

“... we were able to confirm data from a 2-hybrid screen and from co-immunoprecipitation experiments that demonstrated a dynamic interaction between a membrane receptor and a transcription factor.”

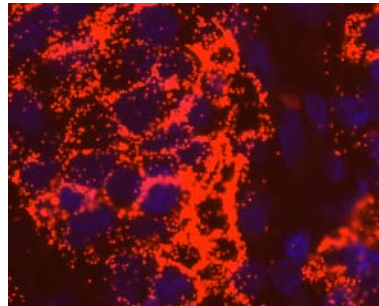
Roxana Pincheira, PhD,  
University of California San Francisco

## 4. STUDY HOMODIMERIC PROTEIN COMPLEXES

Duolink Probemaker enables the study of homodimeric complexes by allowing the use of same species monoclonal antibodies. Simply include Probemaker probes, instead of PLA probes, in a Duolink kit.



Normal tissue

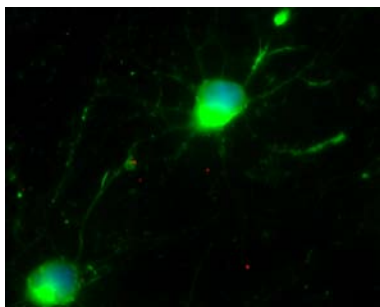


Cancerous tissue

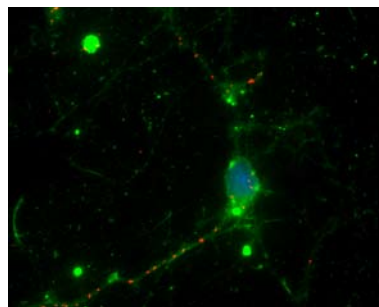
**Fig 5.** Visualization of HER2/HER2 homodimers in breast tissue.  
Red: HER2/HER2 homodimers.  
Blue: DAPI stained nuclei.

## 5. WORK WITH ENDOGENOUS PROTEINS USING ONLY A FEW CELLS

ER alpha/DYX1C1 interaction visualized in cell's neurite extensions. Rat embryonic hippocampal neurons cultured 48 hours with estradiol. Proteins detected at endogenous levels – not seen using Western blot.



Unstimulated control



Estradiol-stimulated

**Fig 6.** Red: ER alpha/DYX1C1 interactions.  
Blue: Hoechst stained nuclei.  
Green: FITC-anti-actin.

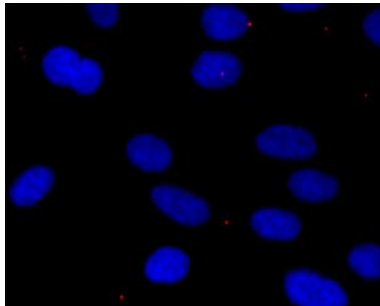
*Reproduced through the courtesy of Human Molecular Genetics. Ref: Massinen et al. Hum Mol Genet. 2009 May 7.*

“... an important tool ... for visualization of novel protein-protein interactions in cell lines and primary cells from different species.”

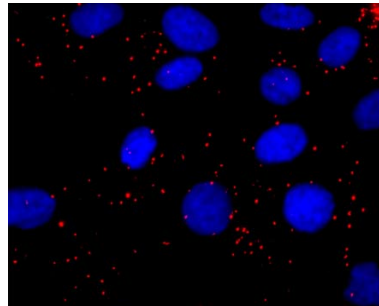
*Hans Matsson, PhD,  
Dept. of Biosciences and Nutrition,  
Karolinska Institute, Sweden*

## 6. DIFFERENTIATE BETWEEN SUB-POPULATIONS OF CELLS

Evaluate phosphorylation levels. Discrete fluorescent signals reveal phosphorylation of PDGF receptors.



Unstimulated control



BB (PDGF ligand)-stimulated cells

Fig 7. Red: phosphorylated PDGF receptors.  
Blue: Hoechst stained nuclei.

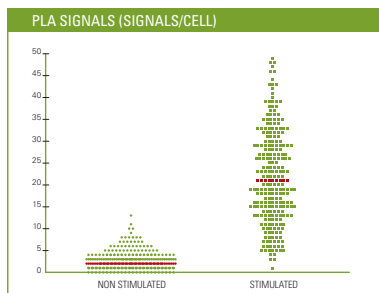


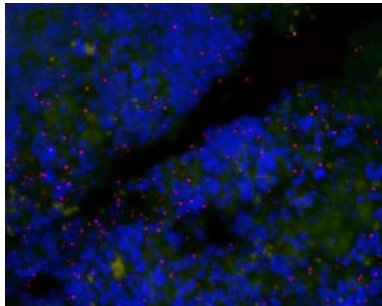
Fig 8. Dedicated Duolink ImageTool generates accurate data.

“... allows visualization of the cellular compartment (nucleus, cytoplasm, etc) in which protein-protein interactions take place.”

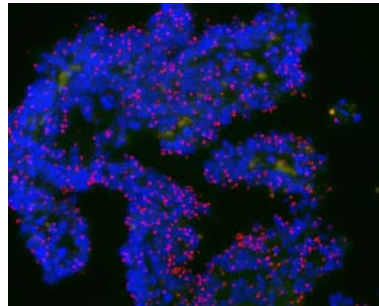
Mark Wade, PhD,  
Gene Expression Laboratory (Wahl Lab),  
The Salk Institute, San Diego, USA

## 7. SEE SUB-POPULATIONS OF CELLS WITHIN TISSUE SAMPLES

Analysis of PDGF receptor in paraffin-embedded choroid plexus carcinoma tissue reveals phosphorylated PDGF  $\alpha$  and  $\beta$  receptor complexes *in situ*.



$\alpha$  receptor



$\beta$  receptor

Fig 9. Red: PDGFR  $\alpha$  or  $\beta$ .  
Blue: Hoechst stained nuclei.  
Green: autofluorescence.

Reproduced through the courtesy of Janna Paulsson, PhD. Dept. of Oncology-Pathology Karolinska Institute. Ref: Koos B, et al. *Am. J. Pathol.* 2009, 175:1631-163.

## 8. CONFIRM CONVENTIONAL CO-IP AND CO-LOCALIZATION RESULTS – IN ONE STEP

Detection of PDGF-R $\beta$ /VEGF-R2 receptor interactions after growth factor treatments of cells.

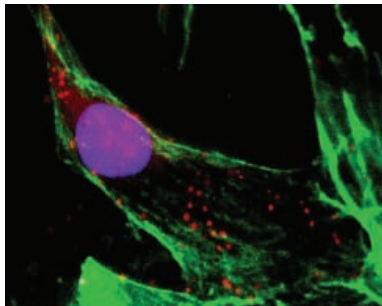


Fig 10. Red: PDGF-R $\beta$ /VEGF-R2 receptor interactions. Blue: Hoechst stained nuclei. Green: Cytoplasm stained with Alexa 488-Phalloidin.

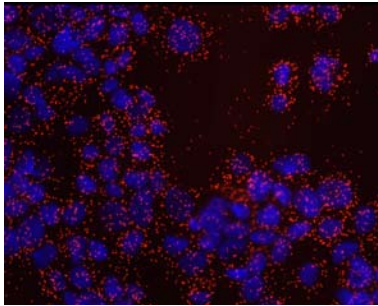
Reproduced through the courtesy of Nature.  
Ref: Greenberg et al. *Nature* 2008 Nov 9.

“... may ultimately prove to be equivalent to FRET, but with the significant benefit of detecting endogenous protein-protein interactions.”

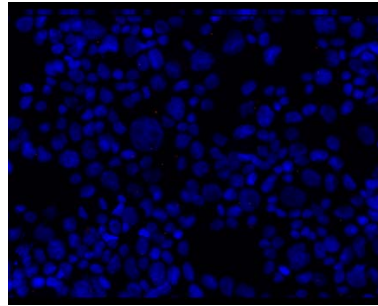
David A. Cheresh, Ph.D.,  
Professor and Vice Chair of Pathology,  
University of California, San Diego,  
Moores Cancer Center

## 9. ACCURATELY QUANTIFY EXPRESSION LEVELS

Discrete fluorescent signals reveal expression of EGF receptor in A431 cells.



Primary antibody against EGFR



Negative control,  
no primary antibody added

Fig 11. Red: EGF receptors.  
Blue: DAPI stained nuclei.

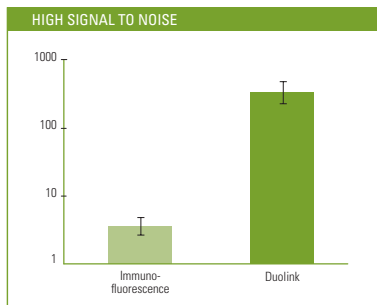


Fig 12. PLA signal-to-noise ratio 100 times higher than immunofluorescence (data derived from comparison with controls - no primary antibody added).

“... a state of the art quantitative protein technology that has furthered our research on human brain tumors above and beyond classical protein techniques.”

*Markus Bredel, MD, PhD,  
Northwestern University, Chicago*

# Results within one day

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## PREPARATION

### PREPARATION AND INCUBATION OF PRIMARY ANTIBODIES AND PLA PROBES.

#### STEP 1.

Fix cells or tissues onto microscope slide, add blocking solution.



Typical starting materials are adherent cells, cytospin preparations or tissue sections on a glass slide, fixed, pre-treated and blocked with a blocking reagent according to the requirements of the primary antibodies used.

#### STEP 2.

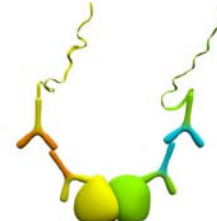
Wash and add two primary antibodies.



Primary antibodies must be of different species and each must recognize two different epitopes on the target molecules.

#### STEP 3.

Wash and add the PLUS and MINUS PLA probes.



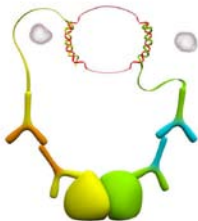
PLA probes are secondary antibodies that bind to the primary antibodies. Each probe is equipped with a unique oligonucleotide.

## DETECTION

### AMPLIFICATION AND DETECTION OF BOUND PLA PROBES.

#### STEP 4.

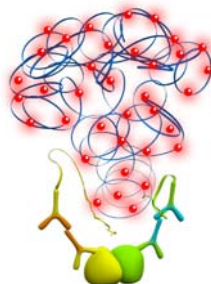
Wash and add Ligation solution.



The Ligation solution, consisting of two oligonucleotides (illustrated as red bands) and Ligase, is added and the oligonucleotides will hybridize to the two PLA probes and join to a closed circle if they are in close proximity (<40 nm).

#### STEP 5.

Wash and add Amplification solution.



The Amplification solution, consisting of nucleotides and fluorescently labeled oligonucleotides (detection probes), is added together with Polymerase. A rolling-circle-amplification reaction generates a concatemeric DNA strand onto which the detection probes subsequently hybridize.

#### STEP 6.

Wash, dry and mount slides.



Use the appropriate Duolink Mounting Medium to preserve and enhance the PLA signals. In order to stain the nuclei use Duolink Mounting Medium with DAPI. For brightfield applications use the nuclear stain included in the kit.

## ANALYSIS

### IMAGING AND QUANTITATIVE ANALYSIS.

#### STEP 7.

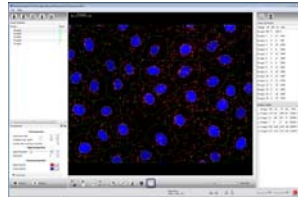
Review and capture images.



The PLA signal is recognized as a fluorescent or chromogenic spot. For fluorescent microscopy, use the appropriate filters for the detection fluorophore used. Export raw data into Duolink ImageTool for objective quantification.

#### STEP 8.

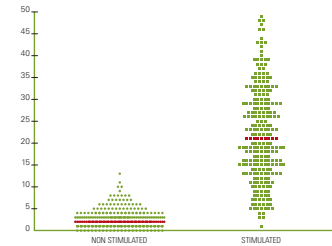
Obtain objective quantification using Duolink ImageTool.



Automatic detection of nuclei and cytoplasmic regions. Compatible with data formats from major microscope vendors including Olympus, Leica, Nikon and Zeiss. Data can be exported into Excel for further evaluation.

#### STEP 9.

Data analysis.

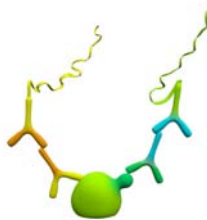


Duolink ImageTool enables single cell statistical analysis of expression levels in tissue or cell populations.

## ADDITIONAL APPLICATIONS

Using the *in situ* PLA technology you can also detect modifications such as phosphorylations or the expression of a single protein.

### DETECT AND QUANTIFY PROTEIN PHOSPHORYLATION



Detection of a modified protein such as a phosphorylated receptor can be done using one primary antibody against the receptor and the other against the phosphorylated site.

### DETECT AND QUANTIFY PROTEIN EXPRESSION



Detection of a single protein with high sensitivity can be done using only one primary antibody. Use PLA probes PLUS and MINUS against the same species.

### DETECT AND QUANTIFY PROTEIN EXPRESSION



Detection of a single protein with high sensitivity and specificity. Use two primary antibodies from different species against different epitopes of the same protein.

# The Duolink solution

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## BUILD YOUR OWN KIT

Each Duolink *in situ* PLA protein detection kit consists of customizable kit components and can be configured to meet specific assay requirements. In addition to the kit components you will also need two primary antibodies raised in different species.



## BUILDING A KIT THAT PERFECTLY MATCHES YOUR NEED IS EASILY DONE IN FOUR STEPS

1. Choose PLA probe PLUS against the species one of your primary antibody has been raised in or Probemaker PLUS
2. Choose PLA probe MINUS against the species the other primary antibody has been raised in or Probemaker MINUS
3. Choose detection reagents depending on your mode of detection, fluorescence or chromogenic
4. Choose the optimal accessories for your Duolink application

All necessary diluent solutions, enzymes and reagents needed to perform the Duolink assay are included in the kit components together with detailed step-by-step instructions.



## CUSTOMIZABLE KIT COMPONENTS

- ▶ **PLA probe PLUS;** anti-Mouse PLUS, anti-Goat PLUS or anti-Rabbit PLUS
- ▶ **PLA probe MINUS;** anti-Mouse MINUS, anti-Goat MINUS or anti-Rabbit MINUS
- ▶ **Probemaker PLUS and MINUS** enables you to “make your own” PLA probes by conjugating the PLA oligonucleotide arms directly to antibodies
- ▶ **Detection Reagents;** Orange, Red, Far red or Green for fluorescence microscopy
- ▶ **Detection Reagents Brightfield** for brightfield microscopy



## RECOMMENDED ACCESSORIES

- ▶ **Wash Buffers** for fluorescence or brightfield – ready-to-use powder for all wash buffers
- ▶ **Control Kit** – reference slides, primary antibody and PLA probes to use as positive controls with fluorescence-based assays
- ▶ **Mounting Medium** with or without DAPI nuclear stain to preserve fluorescence signals
- ▶ **Brightfield Mounting Medium** for chromogenic-based assays
- ▶ **Duolink ImageTool** for image evaluation and result quantification

# How to order

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For information about the Duolink products visit [www.olinK.com](http://www.olinK.com). We strongly recommend that you use the Duolink Kit Wizard, which configures the kit to suit your needs. Orders can be placed directly online or sent via email to [order@olinK.com](mailto:order@olinK.com).

Online purchasing is available in selected countries. For information about your local distributor visit [www.olinK.com/contact/distributors](http://www.olinK.com/contact/distributors).





Olink Bioscience is based on a strong intellectual property portfolio focused towards the development of cutting edge technologies for biomolecular analysis in research and diagnostics. The company's proprietary Duolink product line enables researchers in academia and the pharmaceutical industry to visualize and accurately quantify individual proteins, their interactions and modifications, in unmodified cells and tissues. Such information is crucial to help elucidate disease mechanisms, leading to the development of novel diagnostics and therapeutics. The Duolink line is marketed and sold through a growing distributor network. A second revenue stream comes from the out-licensing of other proprietary technologies to major biotech supply companies.

Olink Bioscience is a privately held company, founded in 2004, and headquartered in Uppsala, Sweden.

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