

“In-cell Co-IP” for visualization of protein interactions *in situ*

INTRODUCTION

Today, many tools are available for studying stable protein-protein interactions. While stable interactions are common in multi-subunit proteins and complexes, transient interactions, expected to control the majority of cellular processes, are more important for studying biologically relevant signal transduction events. Duolink provides the unique capability to study both stable and transient interactions at endogenous expression levels of proteins directly *in situ*.

THE DUOLINK SOLUTION

Duolink is a kit series enabling the use of two primary antibodies for *in situ* immunoassays, thereby bringing the element of dual recognition to localized analysis.¹⁻² The reagents come as pre-optimized reagents for detection and visualization of the Duolink signal and are available in four different fluorescent labels to suit a wide range of applications and instruments, as well as HRP/Novared for brightfield detection (Fig 1).

FASTER RESULTS – NO CELL LYSIS, NO PRECIPITATION, NO WESTERN BLOT

Showing the result of the assayed sample directly *in situ* means there is no need for extra separation and development steps on a gel, as required in a traditional co-immunoprecipitation (Co-IP) experiment. Furthermore, the true cellular and sub-cellular location of the interaction is directly visualized.

The workflow for performing a Duolink experiment is simple and straightforward. It does not require any centrifugation or separation steps and can be carried out using the same equipment as for a standard immunofluorescence experiment. Duolink has been successfully applied to cells grown on slides and in chamber slides, as well as to micro-titer plates, cell arrays, tissue microarrays, and tissue sections on slides. This means it is suitable for high throughput, something that is not possible with traditional Co-IP.

Duolink directly answers the crucial question; does the interaction take place in the cell when the proteins are present in their physiological context, i.e., at physiologically relevant concentrations of all participating proteins in the network.



Fig 1. The Duolink kit series of optimized, simple to use reagents, allows the user to combine any pair of immunofluorescence or immunohistochemistry validated antibodies for direct in-cell detection of protein interaction events. Duolink read-out is performed either with a fluorescent label, for fluorescence microscopy, or HRP for brightfield detection. The resulting distinct spots are derived from individual protein interaction events, which are visualized using a standard microscope.

DUOLINK DETECTS INTERACTIONS NOT DETECTABLE WITH CO-IP

Duolink utilizes fixed cells or tissue ensuring that even transient complexes are intact at the time of measurement. The fixation “freezes” the cells at the desired time-point and allows precise measurement of the dynamics of a cellular process after perturbation by a time-series of fixation. In a recent report the authors studied VEGF-receptor dimerization patterns. While VEGF-C ligand induced heterodimerization between VEGFR2 and VEGFR3 was detected using both Duolink and Co-IP, VEGF-A ligand induced heterodimerization was only found using Duolink.³ This highlights the importance of being able to study transient and weak interactions to fully understand complex biological systems.

VISUALIZATION OF PROTEIN INTERACTIONS – SEEING WHAT IS REALLY THERE

The strong and highly specific amplification of each individual protein-interaction event ensures that every signal is easily visible as a distinct spot in a fluorescence or brightfield microscope. Every signal is physically

PREPARATION



STEP 1. Fix cells or tissues onto microscope slide or microplate.



STEP 2. Wash and add two primary antibodies.

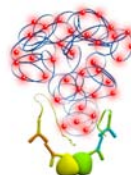


STEP 3. Wash and add the PLUS and MINUS PLA probes.

DETECTION



STEP 4. Wash and add Ligation solution.

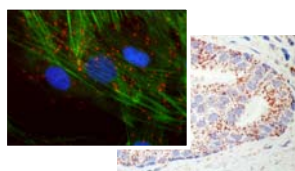


STEP 5. Wash and add Amplification solution.

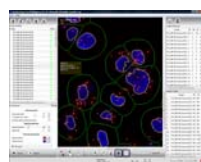


STEP 6. Review and capture images.

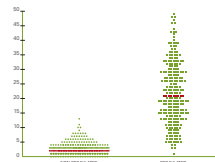
ANALYSIS



STEP 7. Single protein interactions visualized using fluorescence and brightfield respectively.



STEP 8. Obtain objective quantification using Duolink ImageTool.



STEP 9. Data analysis.

Fig 2. Cells or tissue deposited on slides or in micro-titer plates are fixed (Step 1) to preserve activation status and transient interactions. Validated primary antibodies for the targets are added (Step 2) followed by Duolink secondary PLA® probes (Step 3). Detection is performed by forming a reporter substrate based on the proximity of the two primary antibodies used (Step 4) followed by amplification (Step 5). The result is visualized using a standard microscope (Steps 6 and 7). The resulting images can easily be quantitatively analyzed using Duolink Image Tool software (Step 8), which facilitates both average and single cell data analysis (Step 9).

linked to its target and remains in the correct cellular locale, i.e., exactly where the interaction took place. A recent report describes how Duolink was used to visualize the previously undetected interaction between fibroblast growth factor 2 and neuronal cell adhesion molecule in single cell oocytes.⁴ The interaction was only detected in the membrane of oocytes and not in the junction between two oocytes during the two-cell stage.

EASY ANALYSIS AND QUANTIFICATION – RETRIEVE SINGLE CELL DATA

Using the dedicated Duolink Image Tool software it is easy to automatically and accurately quantify signal levels, even for users not familiar with image analysis suites. Quantification can be based on the average number of signals per cell in each region of interest or image, or it can be carried out for each individual cell. Easy analysis of many images from each experiment facilitates calculation of the statistical significance of the result.³

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CONCLUSION

Duolink provides a simple means of simultaneously detecting, visualizing and locating protein-protein interactions in unmodified cells and tissue with exceptional specificity and sensitivity. There is no need to over-express target proteins, and data interpretation is greatly facilitated by countable spots, each representing a unique interaction event. Duolink reagents are available as secondary reagents against different species of primary antibodies, as well as in a mix-and-use format (Probemaker) for direct labeling of any primary antibody.

In addition to all the benefits of traditional Co-IP, Duolink also offers localization information, the ability to study transient and weak interactions, and precise quantification – all in a user-friendly format suitable for high throughput.

REFERENCES

1. Söderberg, O. *et al.* Direct observation of individual endogenous protein complexes *in situ* by proximity ligation. *Nat. Methods*, **3**, 995–1000 (2006)
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