OLINK
BIOSCIENCE

Duolink-"In-cell Co-IP"for visualization of protein interactions *in situ*

Duolink® offers a user-friendly solution for studying protein-protein interactions. Duolink utilizes a pair of antibodies that are capable of quantitatively reporting even weak and transient protein-protein interactions in natively expressing cells either as countable, bright fluorescent spots for a standard fluorescence microscope or via chromogenic detection for brightfield microscopes. It offers all the benefits of traditional coimmunoprecipitation (Co-IP) and western blotting but with better quantitative precision and more information about cell-to-cell variability and target localization. It is also amenable to high throughput in 96 or 384 wells.

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A wealth of tools are available today for studying stable protein-protein interactions. Whereas stable interactions are common in multi-subunit proteins and complexes, transient interactions, which are believed to control the majority of cellular processes, are more important to assay for studying biologically relevant signal transduction events. Duolink provides a unique capability to study both stable and transient interactions at endogenous protein levels 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 preoptimized for detection and visualization of the Duolink signal, and they are available in four different fluorescent labels to suit a wide range of applications and instruments or in HRP/NovaRED for brightfield detection (**Fig. 1**).

Figure 1 | The Duolink kit. 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 readout is performed with either a fluorescent label for fluorescence microscopy or HRP for brightfield detection. The resulting distinct spots are derived from single-molecule protein interaction events, which are visualized using a standard microscope.

Get results faster: no cell lysis, no precipitation and no western blotting

Showing the result for your assayed sample directly *in situ* gives you the information without further need for extra separation and development steps on a gel such as are needed in a traditional Co-IP experiment. Furthermore, the true cellular and subcellular location of the interaction is directly visualized. The workflow for performing a Duolink experiment is simple and straightforward, without any centrifugation or separation steps (**Fig. 2**). A complete Duolink experiment can be performed using the same equipment as for standard immunofluorescence. Duolink has successfully been applied to cells grown on slides, in chamber slides and in microtiter plates and to cell arrays, tissue microarrays and tissue sections on slides. It is amenable to high throughput, in contrast to the low throughput of traditional Co-IP.

Using Duolink directly answers the crucial question of whether the interaction takes place in the cell when all the necessary proteins are present in their physiological context and at physiologically relevant concentrations.

Duolink detects interactions you cannot detect 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 a desired time point and allows one to use a time-series of fixation to obtain precise measurement of the dynamics of a cellular

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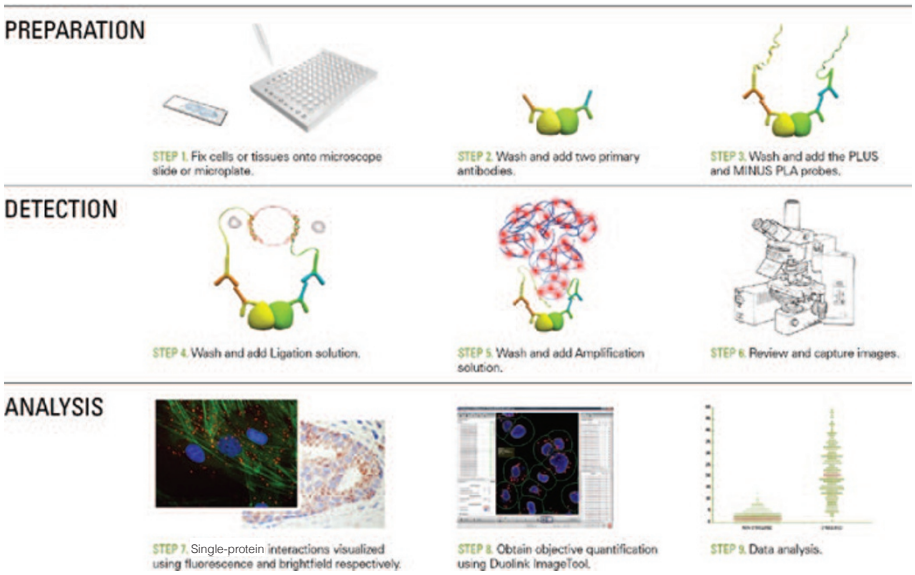

Figure 2 | Duolink in detail. Cells or tissue deposited on slides or in microtiter plates are fixed (**Step 1**) to preserve activation status and transient interactions. Validated primary antibodies for the targets are added (**Step 2**), followed by Duolink secondary PLA probes (**Step 3**). Detection 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**), and the resulting images can easily be quantitatively analyzed using the dedicated Duolink ImageTool software (**Step 8**), which allows both average as well as single-cell data to be used (**Step 9**).

process that has been perturbed. In a recent report, researchers studied VEGF receptor dimerization patterns, and although 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 even transient and weak interactions to fully understand complex biological systems.

Visualization of protein interactions—see what's really there

The strong and highly specific amplification of individual, detected protein interaction events ensures that every signal is easily visible as a distinct spot in a fluorescence or brightfield microscope. Every signal is physically linked to its target and stays in the correct cellular locale as the assayed interaction takes place. A recent report describes how Duolink is 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

The dedicated Duolink ImageTool software available from Olink makes it easy to automatically and accurately quantify the signal levels for each experimental condition either as an average number of signals per cell in each region or image of interest or as individual cell data, even for users unfamiliar with more complex image analysis suites. The ability to easily analyze many images from each experiment facilitates discovery of the statistical significance of the result[3].

Conclusions

Duolink provides a simple means to simultaneously visualize and locate protein-protein interactions in unmodified cells and tissue with exceptional specificity and sensitivity. There is no need to overexpress target proteins, and data interpretation is greatly facilitated by countable spots, each representing a single-molecule event. The Duolink reagents are available as secondary reagents against different species of primary antibodies as well as a mix-and-use Probemaker product for direct labeling of any primary antibody.

Duolink provides you with all the benefits of traditional Co-IP plus the added information of localization, the ability to study transient, weak interactions, and precise quantification, all in a user-friendly format and amendable to high throughput.

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This article was submitted to *Nature Methods* by a commercial organization and has not been peer reviewed. *Nature Methods* takes no responsibility for the accuracy or otherwise of the information provided.

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