Duolink® Technology
Illuminating Insight into Protein Interactions by High Content Screening

Duolink - Areas of Application
Alongside established drug target classes, innovative approaches are addressing previously undruggable target classes such as protein–protein interactions. The new Duolink PLA® technology is an excellent tool to visualize and quantify protein interactions in fixed cells and tissues samples – not only in a single experiment but also in high-content screening applications.

Duolink can be used for pathway analysis as part of the target selection step in the drug discovery workflow to identify a protein associated with a disease with which a potential drug can interact.

In the lead discovery step compound profiling using PLA can be used as part of the hit-to-lead workflow and the key advantage is the ability to provide evidence for target engagement of compound. The PLA technology can be used on primary cells, stem cells, differentiated cells and cancer cell lines in a high-throughput screening format and can be performed in parallel to cell cycle, and compartment specific assessments.

Proximity ligation is a highly specific and sensitive technology to monitor individual proteins, their interactions and post-translational modifications in situ. It provides single molecule resolution and objective and unbiased quantification in cells and tissues on endogenous expression levels. The PLA technology has been optimized for use in an image analysis including spot detection, localisation and quantification.

Duolink Technical Background
Duolink is a kit series in which DNA amplification is coupled to an immunoassay to bring radically new detection possibilities for monitoring protein expression. Duolink In Situ products enable detection, visualization, and quantification of protein events in tissue and cell samples prepared for microscopy.

The in situ PLA technology is essentially an immunofluorescence assay which offers advantages over traditional procedures, as selectivity is increased with dual recognition of target protein(s) by antibody probes, a signal can only be generated when the antibody probes are bound in close proximity, and DNA amplification results in increased sensitivity. The in situ PLA technology is designed to determine the proximity of two epitopes using primary antibodies specific to each epitope. An antibody complex with conjugated oligonucleotides forms at each epitope. A ligation reagent is then added and, if the two epitopes are in close proximity, the conjugated oligonucleotides will hybridize to a connector oligonucleotide. The formation of a circular DNA molecule serves as a template for a rolling circle amplification reaction. After the RCA reaction, appropriately labeled detection oligonucleotides (fluorophores for fluorescence and horseradish peroxidase for brightfield microscopy) are added, resulting in hybridization of 500–1,000 labeled detection oligonucleotides to the repeated sequence encoded in the DNA circle, allowing observation of single protein events, like protein post-translational modifications, protein-protein interactions, and protein expression.

The method can be used to capture a snapshot of the levels of proteins in cells and tissues, the relative activity of membrane receptor complexes at rest and under stimuli, and the degree of post-translational modifications (PTMs) such as phosphorylation of receptors in signal transduction pathways. Furthermore, the Duolink assay workflow is scalable to accommodate single cells to tissue arrays. Finally, a method is now available to meet the demands of studying protein expression with objective quantitation, high specificity, per-cell resolution and scalable to analyze many tissue samples.

Duolink – Advantages in comparison with other technologies

FRET
The time to results using Duolink is much quicker and with lower risk for false positives than with FRET.

With FRET recombinant proteins have to be designed which can take 6-18 months to optimize conditions.
These recombinant proteins are no longer expressed under the endogenous promoter which means they may be overexpressed. Thus, if an interaction is observed with overexpressed proteins then the data becomes less reliable as there may be an interaction solely based on concentration.

There are some other technical challenges in microscopy with FRET such as low signal (brightness) which is not applicable with Duolink® because of the amplification step.

Co-Immunoprecipitation

With Co-IP only primarily high-affinity interactions can be measured. This method is semi-quantitative only. There is a need to overexpress low abundant proteins, therefore it cannot be used to study endogenous protein levels. Co-IP can be used for tissue samples, but not on fresh, frozen or FFPE tissues as Duolink.

Duolink - Benefits

- Visualize individual interactions without having to overexpress proteins.
- Gain high specificity with dual binding of primary antibodies.
- Single molecule sensitivity due to signal amplification.
- Analyse using a standard fluorescence microscope.
- Achieve results in less than one working day.
- Adaptable to High Content Screening in 96 or 384 well plates.

To find a detailed description of the DuoLink technology including application notes and an introductory video, visit sigma.com/duolink

If you would like to be contacted by Sigma-Aldrich or receive additional information on Duolink, please visit: sigma-aldrich.com/duolink-enquiry