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ATLAS ANTIBODIES

MolBoolean™

BEYOND PROXIMITY LIGATION

UNLOCK THE SECRETS OF PROTEIN-PROTEIN INTERACTIONS IN CELLS AND TISSUE

PRODUCT DESCRIPTION

MolBoolean™ mouse/rabbit is a kit for in situ protein proximity analysis in tissue and cells.

The MolBoolean™ assay utilizes a proprietary oligonucleotide setup that enables the simultaneous detection of both free and interacting (~40 nm proximity) fractions for two protein targets (protein A, protein B and interaction proteins AB).

MolBoolean™ Mouse/Rabbit should be performed with the user's primary antibodies of choice (raised in mouse and rabbit). MolBoolean™ relies on anti-mouse and anti-rabbit secondary proximity probes and rolling circle amplification (RCA) as a mean to amplify signal.

A series of molecular steps, performed in the MolBoolean™ assay, incorporates information into the amplified products indicating antibody target engagement of individual versus interacting proteins. This information is converted to fluorescent signals by the binding of detection reporters, allowing visualization on a conventional fluorescent microscope.

An image analysis software is provided to segment and differentiate fluorescent signals, enabling the relative quantification of free versus interacting fractions for the two analyzed protein targets in the sample.

MolBoolean™ is based on the 'boolean logic' for the simultaneous detection of free and interacting protein fractions.

WHY MOLBOOLEAN?

- Complete spatial quantitative analysis of protein-protein interactions by **simultaneous detection** of free and interacting proteins.
- Accurate **quantification by normalization** of interaction data to total target protein levels.
- Biologically relevant data **without the need** for engineered protein expression.
- **1000-fold** increased fluorescence signal by Rolling Circle amplification, allowing detection and quantification of low abundant proteins.
- **Adaptable** to different research needs.
- **Universal** kit that can be used with the customer's choice of primary antibodies.
- **Validated** in both cells and tissue.

The MolBoolean™ kit contains 15 separate tubes. The kit volume is 4.8 ml and covers approximately 120 assays in cells (40 µl/assay) and 60 assays in tissue (80 µl/assay).

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MolBoolean: Overcoming in situ PLA limitations

MolBoolean™ offers several advantages over traditional methods like in situ PLA, addressing key gaps in data interpretation and providing more reliable results:

- **Normalization of Data:** With MolBoolean™, the **number of protein interactions can be normalized to the total number of target proteins**. This is crucial because target protein levels can be influenced by various factors such as cell treatments or disease states. By providing a normalized measure, MolBoolean™ allows for more accurate comparisons between samples.

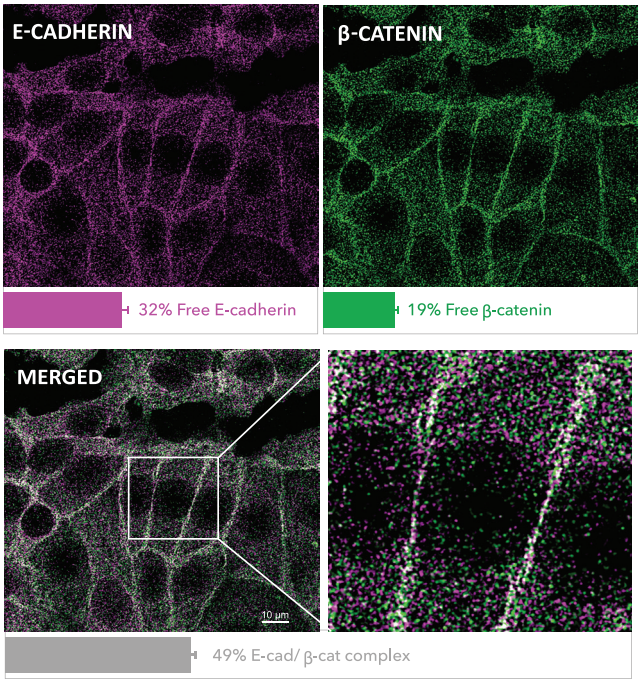
- **Detection of Interacting and Non-interacting Fractions:** Unlike traditional methods, MolBoolean™ can **detect both interacting and non-interacting endogenous fractions for two protein targets in cells and tissues.** The more comprehensive detection capability increases the spatial information that can be extracted from the experiment, allowing researcher to determine locations of positive or negative protein interactions.

- **Consistent Molecular Process:** The Rolling circle products (RCPs) stemming from free or interacting proteins are **generated through the same molecular process steps**. This ensures uniform signal efficiencies, reducing uncertainty during data analysis.

Overall, MolBoolean™ offers a more reliable and comprehensive solution for detecting protein interactions, addressing key gaps in data interpretation, and providing researchers with more accurate insights into molecular interactions in cells and tissues.

Assay	Individual Proteins	Protein Interaction
IHC/ICC	✓	✗
in situ PLA	✗	✓
MolBoolean	✓	✓

MolBoolean™ compared to common protein detection assays.



E-cadherin/ β-catenin MolBoolean staining in MCF7 cells.

MolBoolean™ analysis of the interaction between E-cadherin (magenta) and β-catenin (green) in MCF7 cells, using the monoclonal anti-CDH1 (Cat. AMAb90862) and the polyclonal anti-CTNNB1 (Cat. HPA029159) antibodies from Atlas Antibodies AB. Image shows the relative quantification of free versus interacting protein fractions, indicated by the detection of rolling circle products (RCPs) in either one or two fluorescent channels: 32% free E-cadherin (magenta), 19% free β-catenin (green) and 49% E-cadherin/β-catenin complex (white). Data is normalized to total target protein levels (total RCPs).

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