MolBoolean
BEYOND PROXIMITY LIGATION

Beyond Proximity Ligation: Accelerate Drug Discovery with MolBoolean™

What is MolBoolean?

MolBoolean™ is a new proximity ligation technology for protein-protein interaction (PPI) studies developed by Atlas Antibodies that enables the simultaneous detection of both free and interacting fractions for two protein targets in cells and tissue. MolBoolean™ is a game-changers for the pharmaceutical and biotech industries, enabling new therapeutic strategies, improving precision medicine, enhancing drug discovery, and facilitating the development of better biomarkers.

Beyond Proximity Ligation: Overcoming in situ PLA limitations

Assay	Individual Protein	Protein Interaction
in situ-PLA	✗	✓
MolBoolean	✓	✓

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
- 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.
- Peer reviewed technology

Rivas-Santisteban R, et al, Neuropharmacol. 2024 Nov 27:110242.
Kotliar IB, et al, Sci Adv. 2024 Aug 2;10(31):eado9959.
Raykova D, et al, Nat Commun. 2023 Sep 6;14(1):5450.
Rivas-Santisteban R, et al, Neurobiol Dis. 2023 Nov;188:106341

Applications of MolBoolean

Drug Discovery: Identification of novel targets and compounds, enhanced by the use of PPI assays for screening and mechanism understanding.

Personalized Medicine: Tailored therapeutic strategies based on PPI profiles for individual patients.

Biomarker Development: Discovery of disease-relevant biomarkers for early detection, prognosis, and treatment monitoring.

Improved Drug Efficacy and Safety: PPI-based insights can help refine drug design, increasing therapeutic efficacy while minimizing off-target effects.

Expanded Druggable Space: Insights into how to target traditionally undruggable proteins and their interactions.

By understanding the complex networks of protein interactions, pharmaceutical and biotech industries can develop more effective, targeted treatments that will ultimately lead to improved patient outcomes.

What's Included in the MolBoolean Kit?

The MolBoolean kit contains 15 separate kit tubes and diluents.
1:Blocker (4x); **2:**Diluent (1x); **3:**Probe A (80x); **4:**Probe B (80x); **5:**Circle (40x); **6:**Additive (40x); **7:**Buffer A (10x); **8:**Nickase enzyme (80x); **9:**Buffer B (8x); **10:**Tag oligos (40x); **11:**Ligase enzyme (80x); **12:**Polymerase enzyme (40x); **13:**Buffer C (5x); **14:**Detection oligos (40x); **15:**Buffer D (10x)

The kit volume (4.8 ml) covers approximately 120 assays in cells (40 µl/assay) and 60 assays in tissue (80 µl/assay).

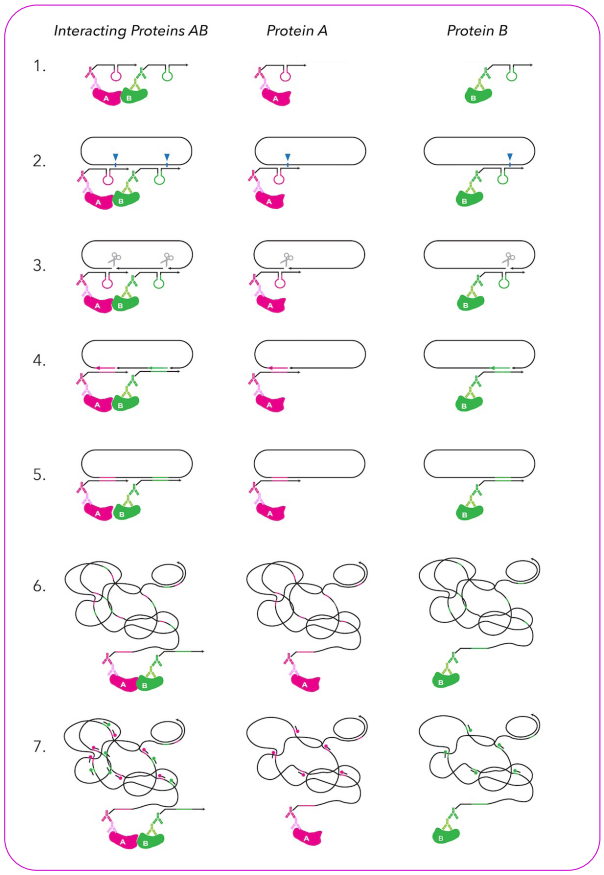
Assay step and workflow

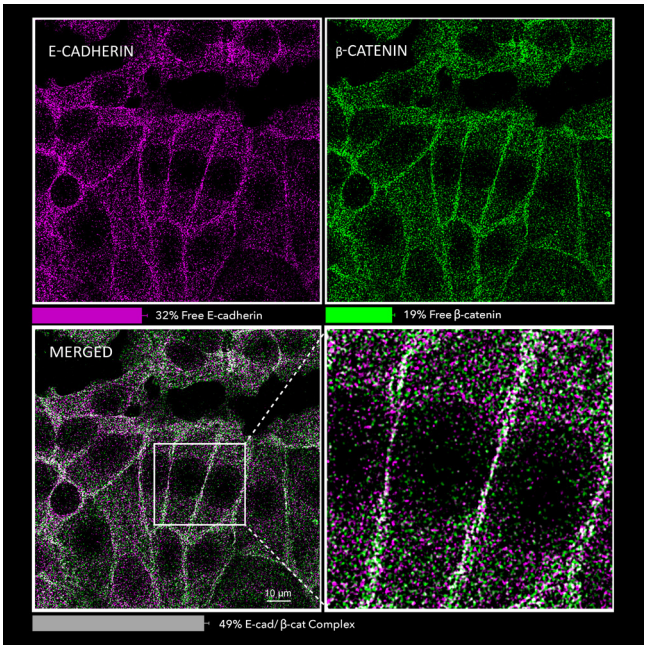
MolBoolean™ Mouse/Rabbit utilizes anti-mouse and anti-rabbit secondary proximity probes and a proprietary oligonucleotide setup that enables the simultaneous detection of both free and interacting (within a proximity of ~40 nm) fractions for two proteins of interest (protein A, protein B and interaction proteins AB).

It uses two different immunofluorescent detection reporters with maximum emission wavelengths at 590 nm (ATTO565, TxRed filter or similar) and at 664 nm (ATTO647N, Y5 filter or similar), respectively.

The MolBoolean™ assay involves seven steps to detect whether the proteins of interest are interacting or present individually:

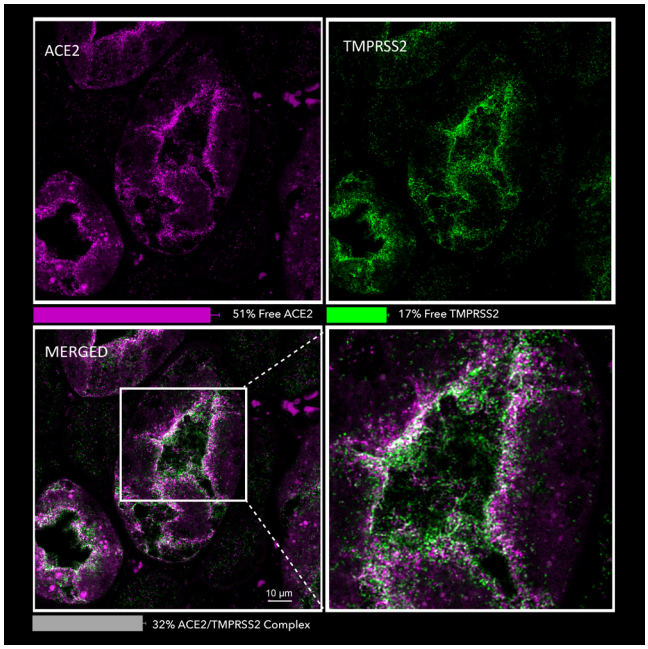
- Step 1: Proximity probe binding to primary antibodies.
- Step 2: Proximity probe arm hybridization to DNA circle oligo.
- Step 3: DNA nicking.
- Step 4: Tag oligo incorporation.
- Step 5: DNA ligation.
- Step 6: Rolling Circle Amplification.
- Step 7: Detection.





E-Cad/ β-Cat MolBoolean staining in MCF7 Cells

MolBoolean™ analysis of the interaction (white) 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 vs 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), 49% E-cadherin/β-catenin complex (white). Data is normalized to total target protein levels (total RCPs).



ACE2/TMPRSS2 MolBoolean staining in human kidney

MolBoolean™ analysis of the interaction (white) between ACE2 (magenta) and TMPRSS2 (green) in human kidney, using the monoclonal anti-ACE2 (Cat. AMAb91259) and the polyclonal anti-TMPRSS2 (Cat. HPA035787) antibodies from Atlas Antibodies AB. Image shows the relative quantification of free vs interacting protein fractions, indicated by the detection of rolling circle products (RCPs) in either one or two fluorescent channels: 51% free ACE (magenta), 17% free TMPRSS2 (green) and 32% ACE/TMPRSS2 complex (white). Data is normalized to total target protein levels (total RCPs).