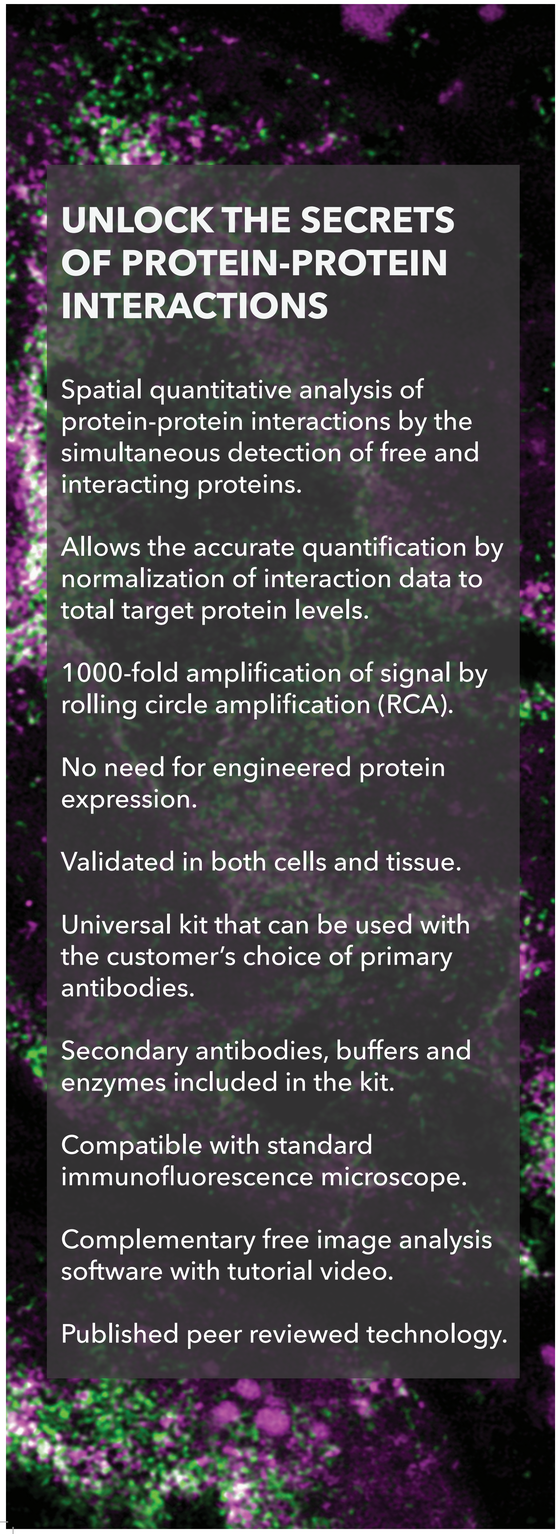
MolBoolean™

BEYOND PROXIMITY LIGATION

by ATLAS ANTIBODIES



UNLOCK THE SECRETS OF PROTEIN-PROTEIN INTERACTIONS

Spatial quantitative analysis of protein-protein interactions by the simultaneous detection of free and interacting proteins.

Allows the accurate quantification by normalization of interaction data to total target protein levels.

1000-fold amplification of signal by rolling circle amplification (RCA).

No need for engineered protein expression.

Validated in both cells and tissue.

Universal kit that can be used with the customer's choice of primary antibodies.

Secondary antibodies, buffers and enzymes included in the kit.

Compatible with standard immunofluorescence microscope.

Complementary free image analysis software with tutorial video.

Published peer reviewed technology.

What is MolBoolean?

MolBoolean™ is a kit for in situ protein proximity analysis in tissue and cells developed by Atlas Antibodies. Unlike traditional methods, it provides spatial quantitative analysis of protein-protein interactions by the **simultaneous detection of free and interacting proteins** (~40 nm proximity).

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

The "Molecular Boolean" logic

The MolBoolean™ assay applies a Boolean logic at a molecular level to distinguish between free and interacting proteins, mapping protein interactions with high spatial specificity.

A B — The **OR logic** identifies either protein A or B independently, signaling that they are present but not necessarily interacting.

AB — The **AND logic** is applied when proteins A and B are interacting; they are detected together as complex AB, indicating a combined "true" interaction.

What's Included in the Kit?

The MolBoolean™ kit includes enzymes (nickase, ligase, polymerase), buffers, oligos for tagging and detection, secondary antibodies and additional reagents such as blocker, diluent, and probes.

The total volume of 4.8 ml, is sufficient for approx. 120 cell assays or 60 tissue assays.

Assay Steps and Workflow

The MolBoolean™ assay follows a two-days 7 steps workflow to determine if target proteins are interacting (AB) or present individually (A, B).

Day 1 (2 hours):
blocking and primary antibodies incubation.

Day 2 (7-8 hours):
probe binding, tagging, amplification, and detection.

(1) Proximity probes bind to primary antibodies attached to the proteins.
(2) Proximity probes link to the DNA circle.
(3) DNA nicking prepares the DNA for tagging.
(4) Tag oligos are added to mark the proteins.
(5) DNA ligation connects the DNA segments.
(6) Rolling circle amplification (RCA) boosts the signal for easier detection.
(7) Detection reveals both interacting and separate proteins.

Why Choose MolBoolean?

MolBoolean™ offers significant benefits in fields requiring complex spatial interaction and quantification data:

Dual Detection Capabilities
Identify both free and interacting protein fractions simultaneously in cells and tissues, giving a comprehensive view of protein behavior.

High Sensitivity
Achieve 1000-fold amplification of signal for exceptional clarity in detecting interactions, allowing visualization and quantification of low abundant proteins.

Compatibility
Easily integrate MolBoolean™ with your existing, validated antibodies and protocols, offering flexibility and saving time in diverse research applications.

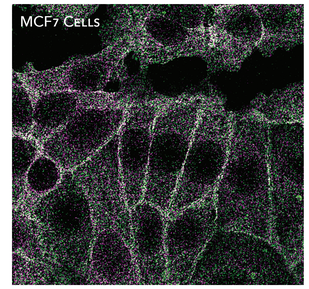
Published Peer Reviewed Technology

Kotliar IB, et al. (2024) **Multiplexed mapping of the interactome of GPCRs with receptor activity-modifying proteins.** Sci Adv. 2024 Aug 2;10(31):eado9959.

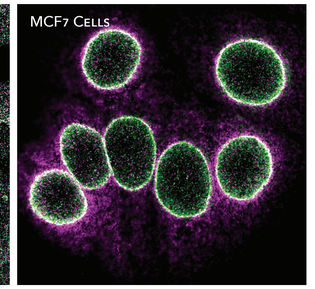
Raykova D, et al, (2023) **A method for Boolean analysis of protein interactions at a molecular level.** Nat Commun. 2022 Aug 13;13(1):4755. Erratum in: Nat Commun. 2023 Sep 6;14(1):5450.

Rivas-Santisteban R, et al, (2023) **Boolean analysis shows a high proportion of dopamine D2 receptors interacting with adenosine A2A receptors in striatal medium spiny neurons of mouse and non-human primate models of Parkinson's disease.** Neurobiol Dis. 2023 Nov;188:106341.

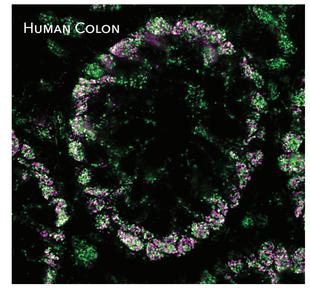
Application in cells and human tissues



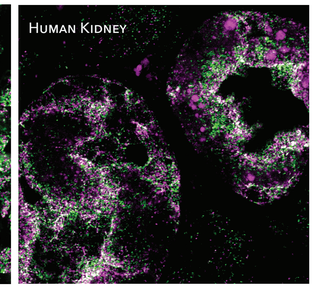
E-cadherin (free) β-catenin (free)
E-cad/β-cat complex



EMD (free) LMNB1 (free)
EMD/LMNB1 complex

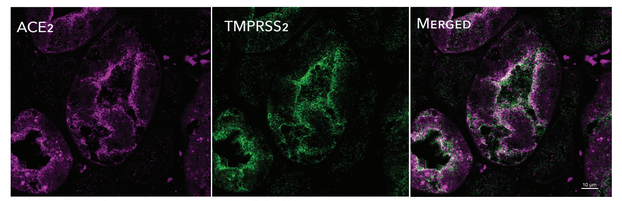


SATB2 (free) HDAC1 (free)
SATB2/HDAC1 complex



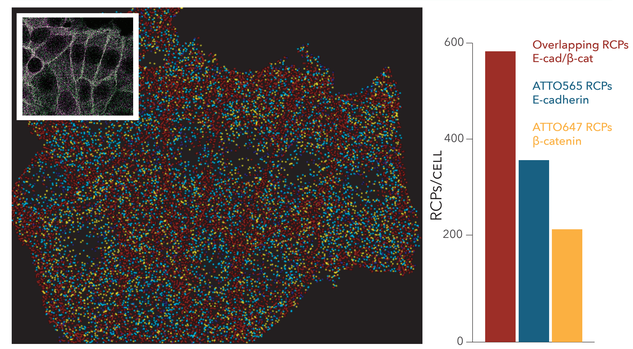
ACE2 (free) TMPRSS2 (free)
ACE2/TMPRSS2 complex

Example of normalization data: ACE2 and TMPRSS2 free vs. interacting protein fractions in human kidney.



ACE2 51% free — TMPRSS2 17% free — ACE2/TMPRSS2 32% complex

Example of image analysis: data segmentation output of E-cad/β-cat in MCF7 Cells



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