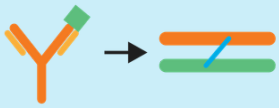


XL-MS: Epitope Mapping

The epitope determines an antibody's mechanism of action, whether it competes with other clones, and how strong its intellectual property position is. XL-MS maps the epitope interface in solution, without sequence engineering. Each cross-link ties a position on the antigen to one on the antibody, so one experiment reports the epitope and the paratope.

What XL-MS measures

A bifunctional cross-linker with a spacer of known length reacts with two residues. The covalent bridge forms only if those residues lie within reach of the spacer at the moment of reaction. After digestion, tandem mass spectrometry identifies the linked amino acids within peptide pairs. The output is a list of residue pairs in spatial proximity, expressed as distance constraints between antibody and antigen.



Antibody peptide above, antigen peptide below, joined by the cross-linker.

Fast results, exhaustive reporting

- **Intact complex data.** High-Mass MALDI MS spectra of free proteins and complex. Analysis of protein complex integrity, stoichiometry and binding.
- **Epitope map.** Cross-linked pairs across the full antigen sequence, with paratope information and coverage stated.
- **Distance-constraint list.** Residue assignments per pair, for structural modelling.
- **Analytical report.** Coverage per enzyme, interface interpretation and epitope characterization.

Why choose CovalX's mapping service?

- **Intact complex verified before mapping.** Every project starts with High-Mass MALDI MS of the free proteins and the assembled complex.
- **Isotopic verification of every cross-link.** Each reported cross-link has a mass-based confirmation built into the experiment, detected by dedicated software.
- **No size restriction on the antigen.** No upper limit on molecular weight or complexity. Compatible with multimeric antigens, hydrophobic proteins, VLPs, full-length mAbs, Fabs, tagged and modified constructs.
- **Patented technology**. Scientists with over two decades in cross-linking MS and protein interaction analysis handle each project.

Delivery

3 to 4 weeks

RUSH options available.

Sample

250µg + 250µg

Antigen and antibody, per epitope.

Resolution

Down to 1-5 aa

Sequence coverage achieved by multi-enzyme digestion.

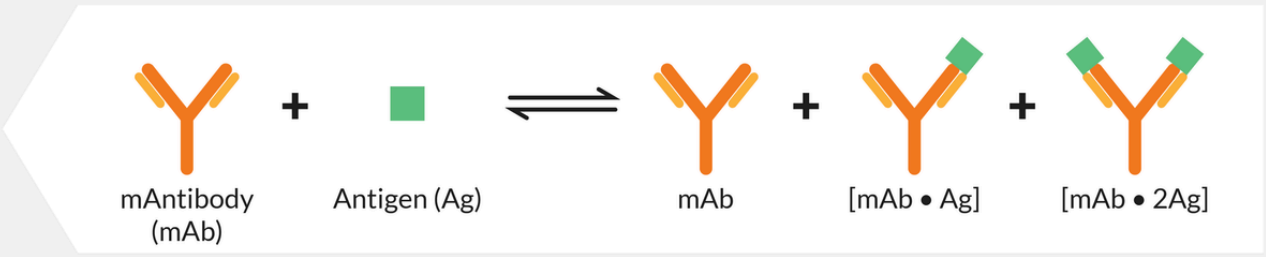
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www.covalx.com | info@covalx.com



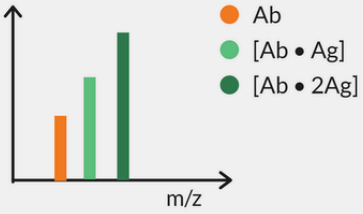
XL-MS: workflow in 3 to 4 weeks

Step 1: Selected Cross-linker & Intact Mass





Direct detection of the Immuno-complexes using High-Mass MALDI ToF. Using MALDI ToF MS equipped with High-Mass system, it becomes possible to rapidly analyze non-covalent interactions in a very broad mass range (0-2000 kDa) with nM sensitivity.

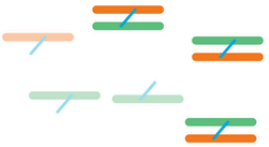


Step 2. Multi-Enzymatic Proteolysis



Purified mAb/Ag cross-linked complex

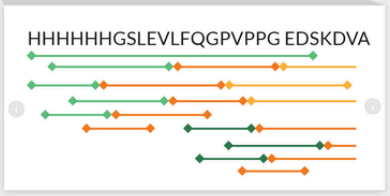
4-5 different enzymes



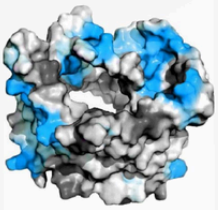
Overlapping cross-linked Ab & Ag peptides

Step 3. nLC-Orbitrap MS/MS analysis





Step 4. Data analysis using interactions software.
Detection of cross-linked peptides (mAb/Ag).



Step 5. High Resolution Epitope Map

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