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White paper

Faster ELISA with better results

One-step sandwich ELISA delivers specific and sensitive results in 90 minutes

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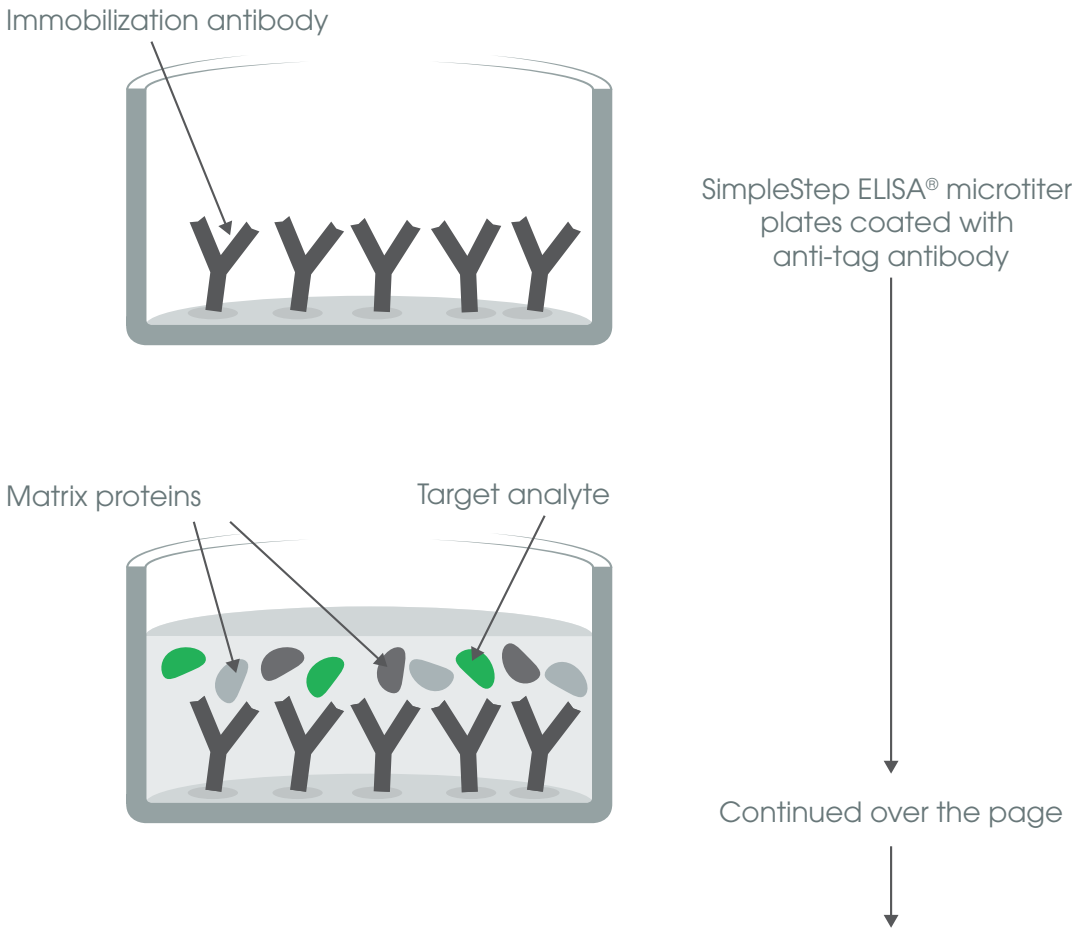
The search for biomarkers for the early detection of human diseases such as cancer, or the identification of novel drug targets in discovery requires an assay that delivers specific, accurate, and sensitive quantification of target proteins. The ideal assay uses raw sample types, such as plasma and serum, which eliminate any bias that results from the sample-preparation process, avoids lengthy and messy sample purification, and reduces the time to results. Protein identification methods like mass spectrometry or antibody-based approaches like western blotting or immunofluorescence can be used, but none of these approaches provides linear quantification of target protein in raw sample types. Only ELISA meets the dual requirements of working with crude biofluids and achieving accurate quantification of the target protein.

The most sensitive and specific format for ELISA is the "sandwich" format, which requires two different antibodies that bind to at least two distinct sites on the target protein. The capture antibody is pre-coated onto a surface, commonly the wells of a 96-well microplate, and selectively binds to the target protein. After a wash step, the detector antibody, which is modified with a detection tag, is added and binds to a second site on the target protein, forming a sandwich complex. However, as a conventional sandwich ELISA may take three hours or more to complete, less time consuming approaches are in demand.

One novel way to speed up an ELISA is to use a homogeneous format where in the antibody/analyte sandwich complex is formed in solution in a single step. SimpleStep ELISA® technology uses a sandwich ELISA format with a novel, streamlined protocol. In a single step, the complete sandwich complex forms in the well and is anchored to the plate with an immunoaffinity tag. Only one incubation and one wash step are required, compared to multiple incubation and wash steps for a conventional ELISA (Figure 1).



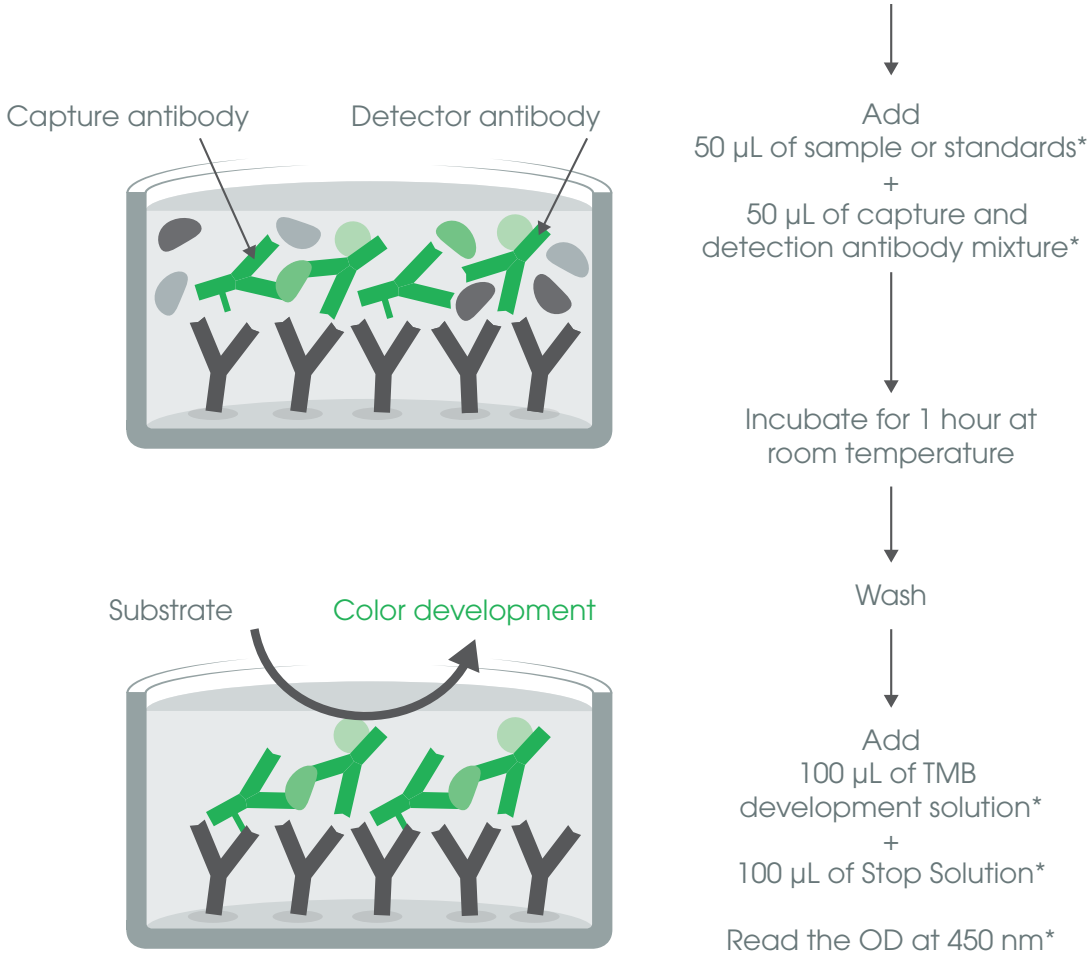


Figure 1.
In SimpleStep ELISA kits, an analyte/capture and detector antibody sandwich complex is formed in solution and binds to the microplate via an affinity tag attached to the 'capture' antibody in the sandwich pair.

*Volumes may vary. Refer to the protocol booklet

One-Step Immunoaffinity Capture Technology

Most ELISA formats make use of the high affinity interaction between biotin and streptavidin to capture or detect the antibody sandwich complex. The presence of biotin in commonly used sample matrices, such as plasma, cell culture media and milk, can interfere with capture or detection of the antibody sandwich complex and complicate data interpretation. Sample dialysis can eliminate biotin, but adds a lengthy step to the protocol. By utilizing a proprietary immunoaffinity technology that uses no streptavidin or biotin, interference from biotin present in the sample is eliminated.

Specificity for target proteins in complex biological samples

Reliable ELISA performance requires the careful selection of the antibody pair that binds to the target protein to create the sandwich complex. Screening many antibodies for pair performance is essential in order to identify a highly specific antibody pair that can then be used in protein-dense solutions such as plasma and serum. To identify a successful antibody pair, individual antibodies are tested pairwise in an ELISA matrix, such that each antibody is tested with other candidate antibodies as both a capture antibody and detector antibody. Only antibody combinations that give the highest overall signal are used for further development. For example, CXCL2/MIP-2 alpha, which has been linked to breast cancer metastasis and chemoresistance, was measured against pairs of RabMAb® recombinant antibodies in order to select for optimal performance (Figure 2).

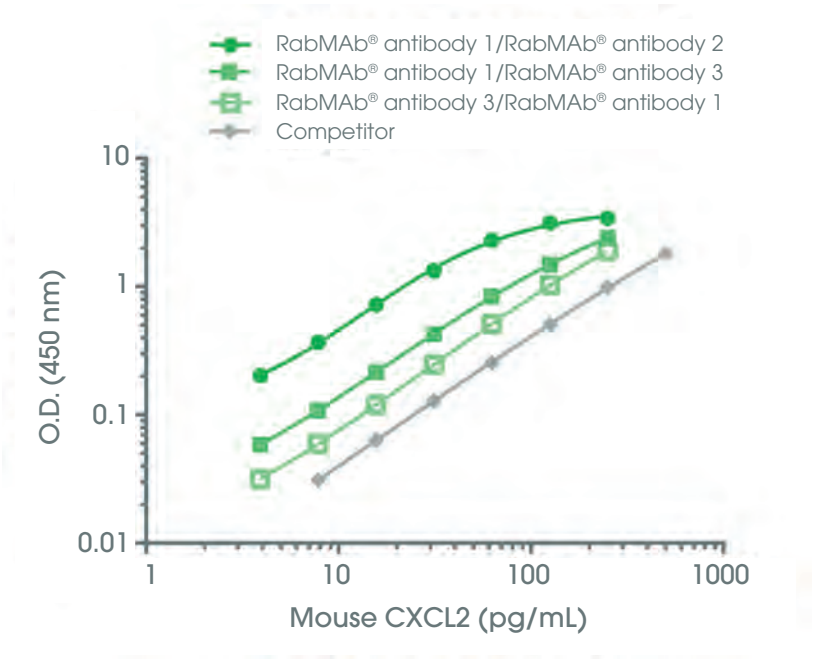


Figure 2.
Antibodies are selected for optimal performance in ELISA. In this example, three of Abcam's RabMAb® antibody pairs against the mouse chemokine CXCL2/MIP-2 alpha show excellent sensitivity relative to a competitor's standard ELISA. Superior sensitivity is an important factor for this chemokine since CXCL2 levels are typically less than 20 pg in undiluted mouse serum.

After the initial screen, the performance of each pair is further characterised in the appropriate biological matrix such as plasma, serum and tissue lysates. Recovery experiments, where purified target protein is spiked in the matrix and linearity studies of sample dilutions, are performed to ensure that the antibody pairs of the ELISA are fit for purpose. This ensures that the antibody pairs recognize not only purified recombinant protein, but also native target protein.

To further verify the specificity of an ELISA kit, a series of related proteins or family members is analysed to evaluate cross-reactivity. For example, to confirm the specificity of mouse CXCL2 SimpleStep ELISA kit, specificity was tested by measuring 2 µg/mL of purified solutions of CXCL3 and CXCL1, which share 82% and 63% amino acid identity with CXCL2 respectively. The signal measured for the related family member proteins was similar to background absorbance values. To determine species reactivity, mouse, rat, goat and pig CXCL2 were assayed. Less than 3% reactivity was determined for all of the species measured, confirming the assays' species specificity.

Improved sensitivity without interference

The use of a homogeneous format improves sensitivity compared to using the same antibody pairs in a conventional, multi-step, sandwich ELISA format. The antibody sandwich is rapidly formed in solution before binding to the plate. As this reaction happens quickly, co-incubation of samples and antibodies for longer than the recommended one hour protocol does not typically yield an increase in sensitivity. For example, CXCL16, part of a chemokine axis implicated in metastatic tumour progression in breast, prostate, and lung cancers, showed increased sensitivity using a homogenous format (Figure 3). Similarly, increased sensitivity was seen for Enolase 1 (ENO1), which is expressed in a wide range of cancer tissues and is being investigated as a potential biomarker for tumour prognosis.

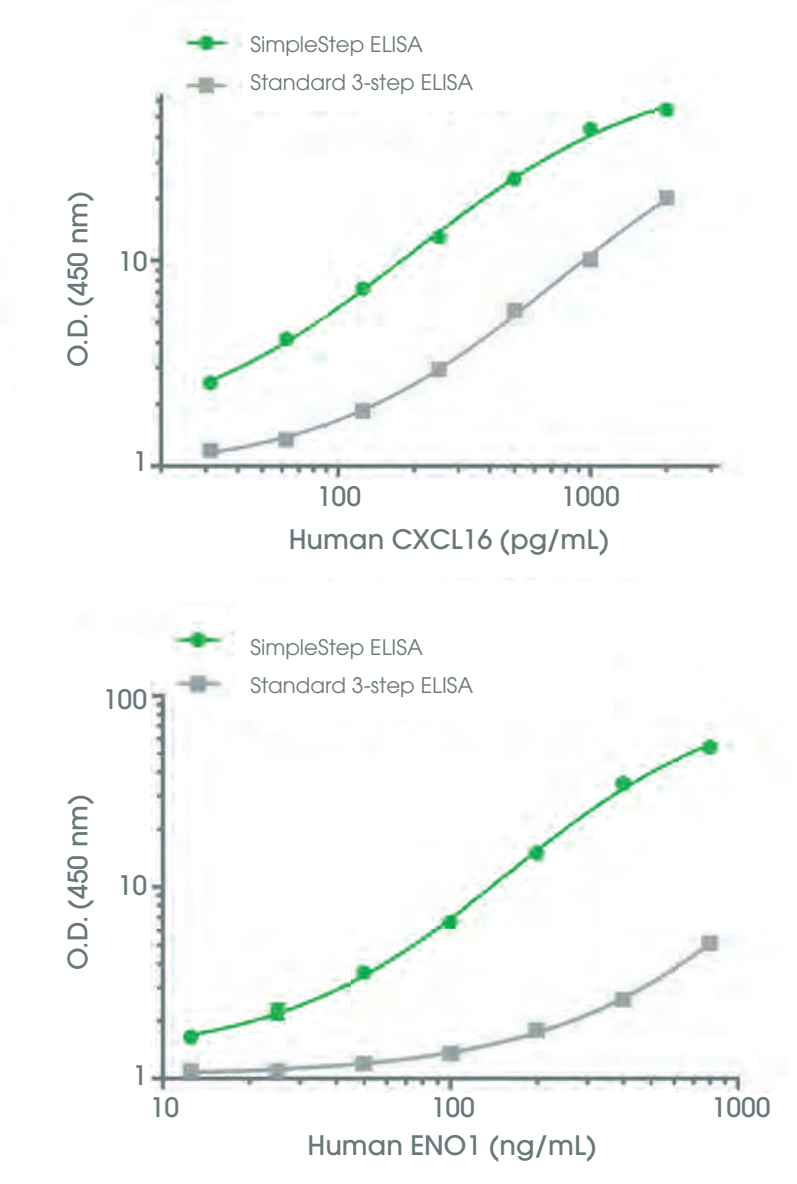


Figure 3.
Sensitivity and range were compared for two antibody pairs in a SimpleStep ELISA or a conventional multiple-step sandwich ELISA format.

In summary, faster ELISA results can be achieved with the use of a single incubation step combined with highly sensitive and specific antibody pairs. In addition, biotin interference is eliminated through the use of a unique immunoaffinity tag. Finally, this approach enables the use of many different sample types without the need for expensive instrumentation or specialized training.

James Murray leads Abcam's SimpleStep ELISA development team in Eugene, Oregon, U.S.A. He holds a PhD in Biochemistry and Molecular Biology from the University of Leeds, UK.