

ELISA

MABTECH

Highly sensitive quantification of cytokines and immunoglobulins

Suitable methods for the detection and quantification of cytokines and other effector molecules are required in order to understand their role and function in health and disease. Similarly, the ability to detect and monitor antigen-specific antibodies is essential for the diagnosis of many diseases, including infections, allergies, and autoimmune disorders, as well as for the development of vaccines.

The enzyme-linked immunosorbent assay (ELISA) is the method of choice for many of these types of applications. Combining the use of well-defined antibodies and reagents with a simple procedure, the ELISA provides a highly sensitive and specific method for measuring almost any analyte. The assay is also very versatile, enabling a range of approaches from the testing of individual samples to fully automated high-throughput screening. As a result, the ELISA has become one of the most commonly used assays both in research and in clinical diagnostics.

Sandwich ELISA

Mabtech has developed ELISA kits to detect a range of cytokines and other analytes derived from humans and several animal species (Table). These kits are all based on the sandwich ELISA technique, which guarantees high sensitivity and specificity.

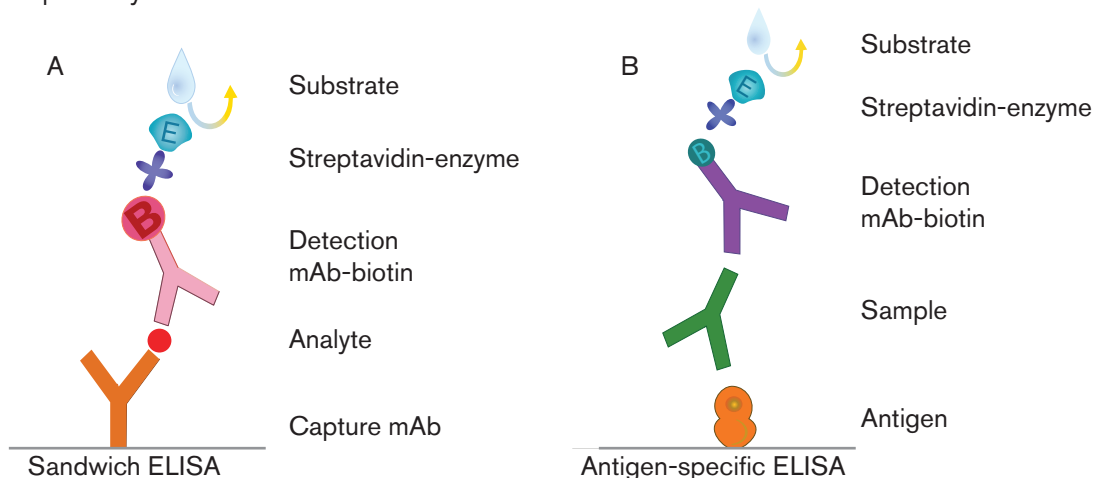


Figure 1. Principle of the sandwich ELISA assay (A) and antigen-specific ELISA (B).

As illustrated in Figure 1A, a monoclonal antibody (mAb) specific for the analyte of interest is used to coat a 96-well ELISA plate. The samples to be analyzed are then added in parallel with a serially diluted standard containing a known concentration of analyte. Detection of bound analyte is achieved by the addition of a biotinylated detection mAb followed by Streptavidin-conjugated enzyme (e.g. ALP) and finally an appropriate colorimetric substrate. The resultant color change is directly proportional to the amount of analyte present in the sample and can be quantified using an ELISA reader. A typical result is shown in Figure 2.

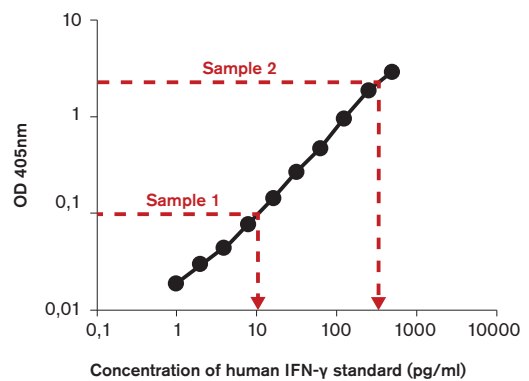


Figure 2. ELISA result. The IFN- γ concentrations of sample 1 and 2 are obtained by comparing their respective OD values to those of a standard curve with known IFN- γ concentration. In this example, sample 1 and 2 were shown to contain 12 and 250 pg/ml IFN- γ , respectively.

Antigen-specific ELISA

The presence of antigen-specific antibodies, for example in serum, plasma, or other biofluids, is typically demonstrated using an indirect ELISA (Figure 1B). With this approach, antigen is first immobilized on an ELISA plate and the sample is then added. The presence of antigen-specific antibodies is revealed by adding a secondary anti-immunoglobulin (Ig) antibody conjugated to an enzyme (please see back cover for a list of available secondary detection reagents); for extra sensitivity, the secondary antibody can be biotinylated and used in combination with Streptavidin-enzyme. Depending on the choice of secondary reagent, the assay can be designed to detect specifically only antibodies of a certain isotype or IgG subclass. The indirect ELISA provides a general tool for measuring antigen-specific antibodies and is the basis for many clinical diagnostic assays in which the immobilized antigen may be a viral or bacterial antigen, an allergen or an autoantigen.

Alternatively, the sandwich approach (Figure 1A), with anti-Ig antibodies for capture and detection, can also be used to quantify the Ig concentration in samples in an isotype- or subclass-specific manner.

Subclass-specific Ig reagents

Human	Mouse
IgA1	IgG1
IgG3	IgG2a
IgG4	IgG2b
	IgG3

Heterophilic antibodies

When human serum and plasma samples are analyzed by ELISA, heterophilic antibodies (human antibodies that react with animal immunoglobulins), which are present in a majority of samples, can give rise to cross-linking between the capture and detection antibodies thus causing false-positive results. To prevent interference by heterophilic antibodies, Mabtech has developed a unique ELISA diluent. This diluent is included in the ELISA^{PRO} kits and can also be purchased separately.

Mabtech ELISA development kits

Human Monkey Mouse

Apolipoproteins

ApoA1	ApoA1	
ApoB	ApoB	
ApoD		
ApoE	ApoE	
ApoH	ApoH	
ApoJ		

Immunoglobulins

IgA	IgA	IgA
IgE		
IgG	IgG	IgG
IgM		IgM

Cytokines

GM-CSF	GM-CSF	
Granzyme B		
IFN- α (subtype 2)		
IFN- α pan	IFN- α pan	
IFN- γ	IFN- γ	IFN- γ
IL-1 β		
IL-2	IL-2	IL-2
IL-4	IL-4	IL-4
IL-5	IL-5	IL-5
IL-6	IL-6	
IL-8 (CXCL8)	IL-8 (CXCL8)	
IL-10		IL-10
IL-12/-23 (p40)	IL-12/-23 (p40)	IL-12/-23 (p40)
IL-12 (p70)		IL-12 (p70)
IL-13	IL-13	
IL-17A	IL-17A	IL-17A
IL-21		
IL-22		
IL-23	IL-23	
IL-31		
MIP-1 β	MIP-1 β	
Perforin	Perforin	
TGF- β 1 (Latent)	TGF- β 1 (Latent)	
TNF- α	TNF- α	

Many systems are also available as ELISA^{PRO} kits with pre-coated strip-plates

Other species

Rat	Cow	Dog	Horse	Pig	Sheep
IFN- γ	IFN- γ	IFN- γ	IFN- γ	IFN- γ	IFN- γ
	IL-4				IL-4
	IL-8	IL-8			

Mabtech ELISA kit formats

Mabtech has for 25 years developed and manufactured high quality monoclonal antibodies suitable for ELISA in a variety of species and offers two different ELISA kit formats:

ELISA development kits

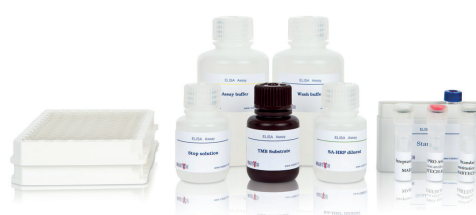
Mabtech's ELISA development kits are adaptable kits suited for researchers who want to set up their own assay. Development kits provide a cost-effective solution and the flexibility to tailor the assay to your needs. ELISA development kits are available in 6 or 20 plate format.



- Capture mAb
- Detection mAb-biotin
- Streptavidin-ALP or -HRP
- Recombinant standard

ELISA^{PRO} kits

Mabtech's ELISA^{PRO} kits provide the necessary reagents to conveniently quantify the analyte of choice. ELISA^{PRO} kits feature pre-coated strip-plates for reduced assay time and minimized assay variability. ELISA^{PRO} kits are available in 2 or 10 plate format.



- Pre-coated strip-plates
- Detection mAb-biotin
- Streptavidin-HRP
- Recombinant standard
- TMB substrate
- Buffers, diluents etc.

Secondary detection reagents

- Goat anti-human IgA-ALP
- Goat anti-human IgG-ALP
- Goat anti-human IgM-ALP
- Goat anti-mouse IgA-ALP
- Goat anti-mouse IgG-ALP
- Goat anti-mouse IgG+IgM-ALP
- Rabbit anti-mouse IgM-ALP
- Goat anti-rabbit IgG-ALP
- Goat anti-rat IgG-ALP

We continuously expand our product portfolio, please visit www.mabtech.com for a complete product listing.