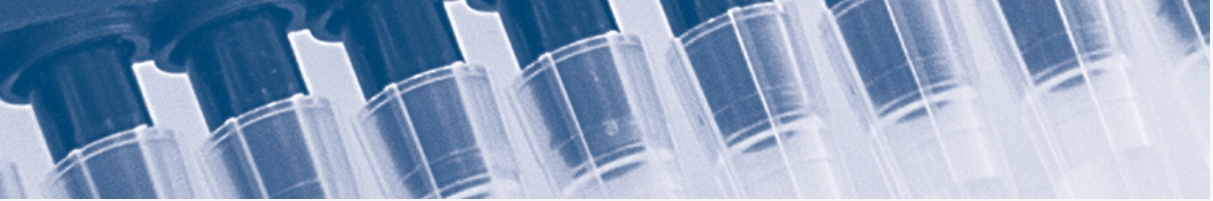

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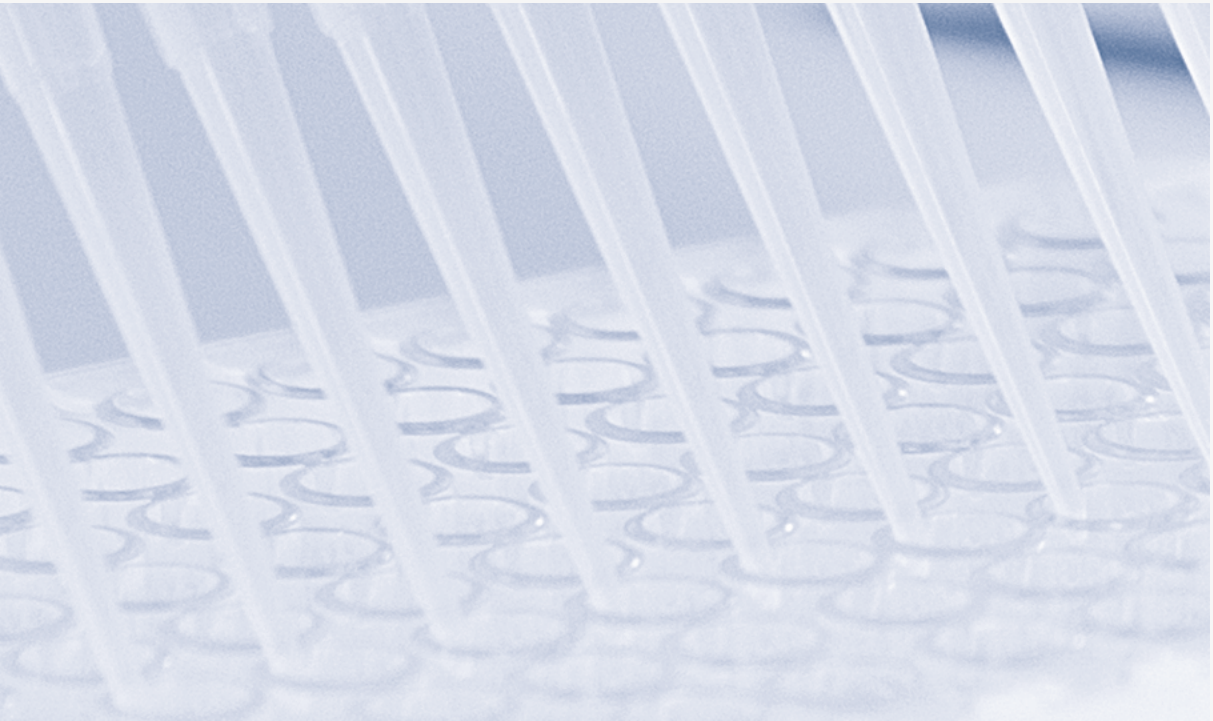
ELISA

Basics and Technical Tips



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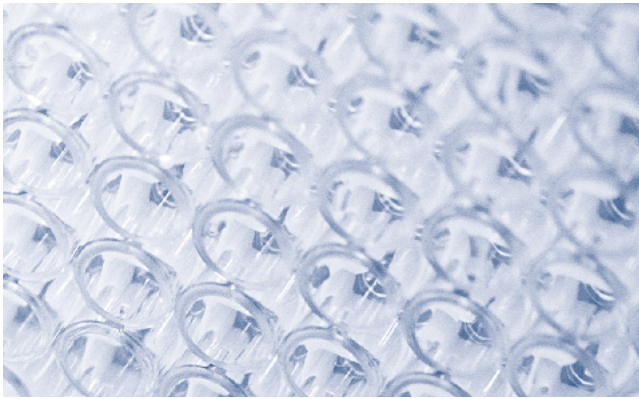

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DEFINITION

ELISA: Enzyme-Linked Immuno-Sorbent Assay

- First described in 1971 by Engvall and Perlmann.
- Characteristic feature of an ELISA: Antigen is directly coated to a well.
- Three main different types of ELISA are currently available.

Why is an ELISA a great research tool?

- A biochemical test that detects and measures antibodies in your blood and antibodies related to certain infectious conditions. ELISA tests are mainly used in immunology.

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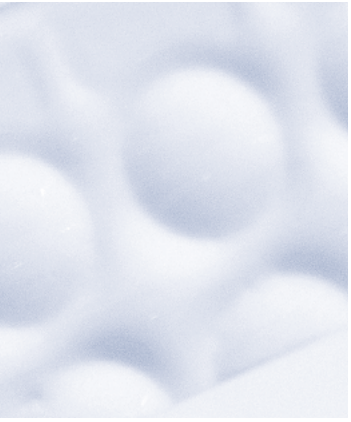
ELISA TYPES



The ELISA technique is divided into:

- **Direct ELISA:**
  The antigen is immobilized on the ELISA plate. The primary antibody carries the label.
- **Indirect ELISA:**
  The antigen is immobilized on the ELISA plate. The secondary antibody carries the label.
- **Sandwich ELISA:**
  Two primary antibodies (for capture and detection) embed the antigen, forming a "sandwich." The complex is then recognized by a secondary labelled antibody.

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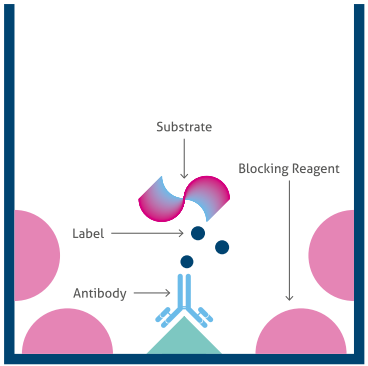
ELISA TYPES

Direct ELISA

- The antigen is immobilized on the ELISA plate.
- The primary antibody carries the label.
- Remaining surface blocked.
- Detection: Dependent on label.

Side view of an ELISA Well.



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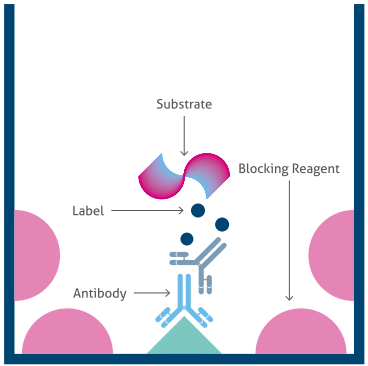
ELISA TYPES

Indirect ELISA

- The antigen is immobilized on the ELISA plate.
- The secondary antibody carries the label.
- Remaining surface blocked.
- Detection: Dependent on label.

Side view of an ELISA Well.



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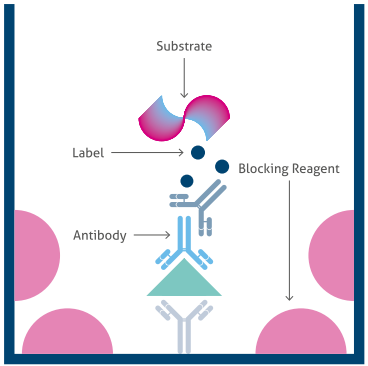
ELISA TYPES

Sandwich ELISA

- Two primary antibodies.
- Capture antibody is bound to the well.
- Remaining well surface blocked.
- Antigen bound by capture antibody and detection antibody.
- Detection: Dependent on label.

Side view of an ELISA Well



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ELISA TYPES

Advantages & Disadvantages

	Direct ELISA	Indirect ELISA	Sandwich ELISA
Costs	+/-	-	-
Cross-reactivity	--	--	--
Availability	+	+	+
Adsorption effects of Antigen	-	-	+
Signal amplification	-	+	+
Assay time	+	-	-

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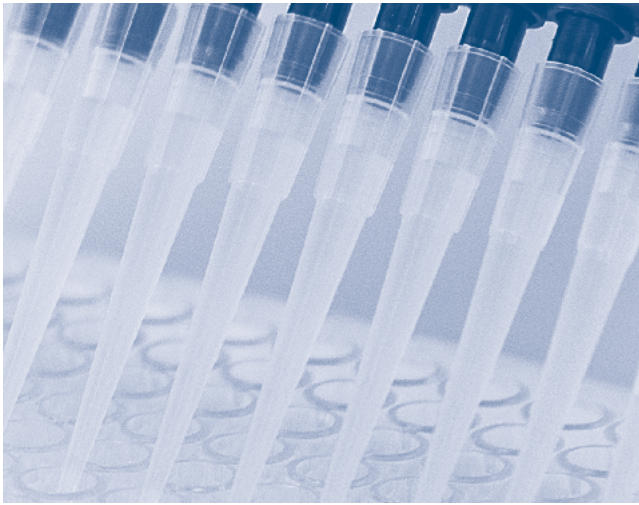
ELISA FACTORS

Factors That Influence an ELISA

ELISA Factor	Factor Characteristics
Plate	Material, type.
Washing	Buffer type, frequency, time, intensity, cross-reactions.
Target-of-interest	Conformation, epitope, matrix effects.
Antibodies	Specificity, concentration, cross-reactions.
Substrate	Sensitivity, lot-to-lot variation.



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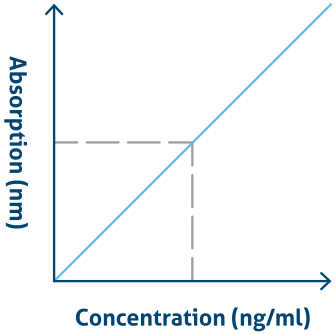
ELISA RESULTS

- The standards are specified on the x-axis.
- The reading of each standard is specified on the y-axis.
- This standard curve is used to determine the unknown concentration.
- The results should be in the range given by the kit manufacturer.



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FAQs

What Is The Purpose Of Different Controls?

Positive Controls	Negative Controls	Zero Blank & Chromogen Blank	Chromogen Blank Useful When
• Standard. • Sample positive control (some companies provide).	• Zero standard. • Chromogen blank.	• OD>0.25 (ELISA dependent) for zero blank means high background. • Comparing zero blank and chromogen blank helps to find the likely cause.	• Background caused by reservoir. • Background caused by metal. • Background caused by chromogen.

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TROUBLESHOOTING

No/Low/High Signal	
Potential Source	**Possible Solution**
Too short/long incubation time.	Incubate following manufacturer's instruction.
Too much/not enough substrate.	Follow manufacturer's instruction.
Poor sample preparation.	Ensure accurate sample type and preparation.
Too harsh handling of plates.	Ensure gentle washing.
Antibody concentration.	Titrate antibody concentration.
Target-of-interest not suitable for assay detection limit.	Double check detection limit of ELISA/ concentrate samples.

Good Standard But No Signal In Samples?
No fresh sample or sample storage incorrect.
Validated sample type?
Many cytokines in normal serum are difficult to detect.

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CONTACT US

Proteintech Group
US Head Office
proteintech@ptglab.com

Proteintech Europe
United Kingdom
europe@ptglab.com

Proteintech
China Office
service@ptglab.com

Support
Available 24 hours via Live Chat and 9–5 (CDT) via phone.

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