abbexa®

do better science

Distributed by:

CliniSciences Group

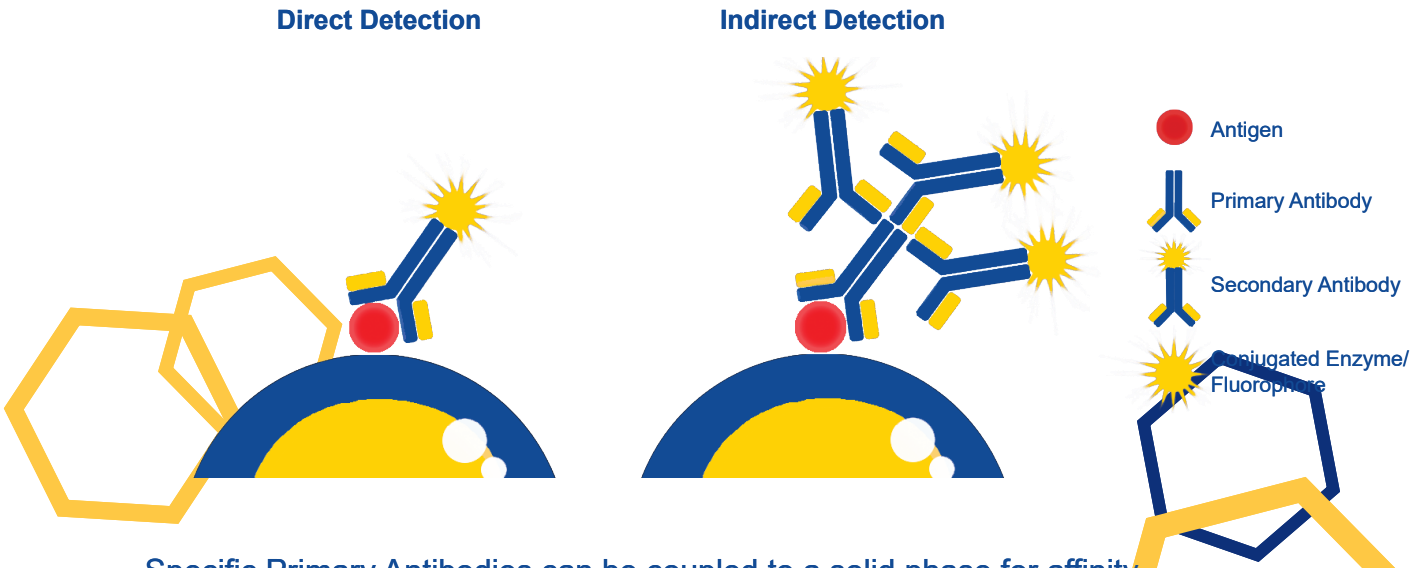
Primary Antibodies

abbexa®

A wide range of Primary Antibodies are available that specifically bind with high affinity to a protein or organic molecule for purification, detection or quantification. These are provided from a variety of hosts including mouse, rat, rabbit and goat, targeting different species accurately, and producing definite and reliable results.

Primary Antibodies can be used to measure changes in the levels of analytes and modifications such as glycosylation, methylation and phosphorylation. Additionally, they can detect specific biomarkers to help understand diseases. Certain Primary Antibodies are supplied already conjugated to various labels for detection in different applications and settings.

Primary Antibody, Secondary Antibody, Antigen, Conjugate Enzyme/Fluorophore



Specific Primary Antibodies can be coupled to a solid phase for affinity purification of target proteins. Proteins can also be isolated using immunoprecipitation studies on identity, structure and expression. Variations of this technique, such as Chromatin-induced Immunoprecipitation (ChiP) provides information on protein-DNA interactions.

Antibodies are required in applications such as Western blotting following fractionation by SDS-PAGE for the detection of analytes, immunohistochemistry and immunocytochemistry (IHC/ICC) to locate the antigen and cytometry for cell analysis. ELISAs are used as the main way to quantify levels of analytes.

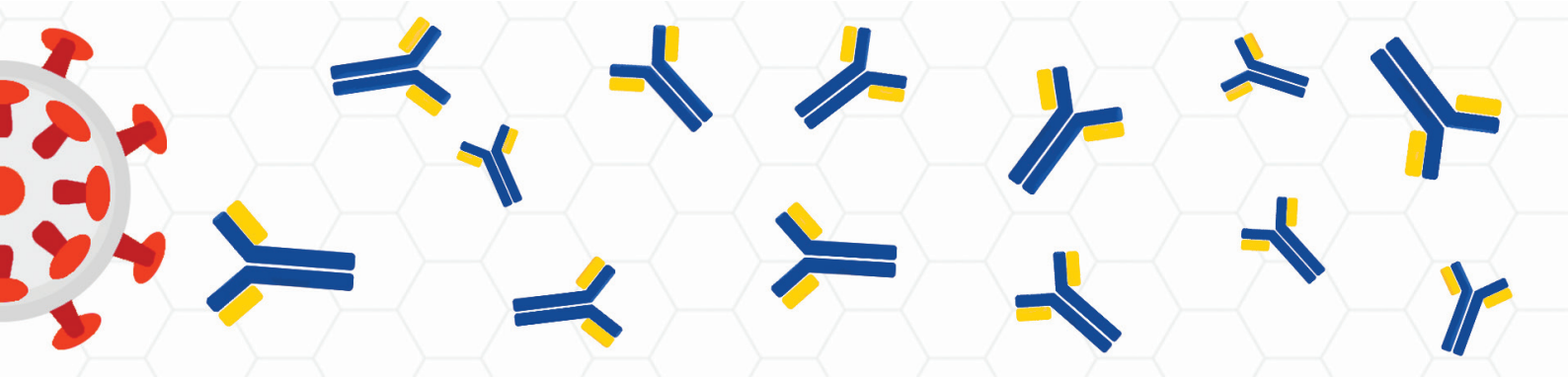
More information can be found in the ELISA section.

Primary Antibodies

abbexa®

The company offers monoclonal and polyclonal Primary Antibodies. Monoclonal antibodies are homogenous antibodies raised in cell culture against a single epitope. This production strategy results in identical antibodies; those with high specificity are selected for mass production.

The recognition of a single epitope reduces the chances of cross-reactivity with other proteins, minimising the background signal in samples. Results from experiments using monoclonal antibodies are highly reproducible, providing the conditions are kept constant.



Polyclonal antibodies are manufactured following the inoculation of a suitable mammal, usually a rabbit or goat, with the required antigen which induces the production of specific immunoglobulins by B-lymphocytes.

These are purified from the serum resulting in antibodies that will recognise and bind multiple epitopes on the antigen.

The benefit of Polyclonal Antibodies is their ability to bind to multiple epitopes. This will amplify the signal dramatically. This can be useful for target proteins with low expression levels.

Polyclonal antibodies are usually inexpensive. Becasue they bind to multiple epitopes on the target, they are able to withstand more alterations in the target. They are also more resistant to environmental changes and conditions. The immunogen sequence should be checked to ensure cross-reactivity does not influence resutls.

For most applications a polyclonal antibody is suitable; in some cases, such as when highly specific and consistent results are required, a monoclonal antibody may be a better choice.

Secondary Antibodies

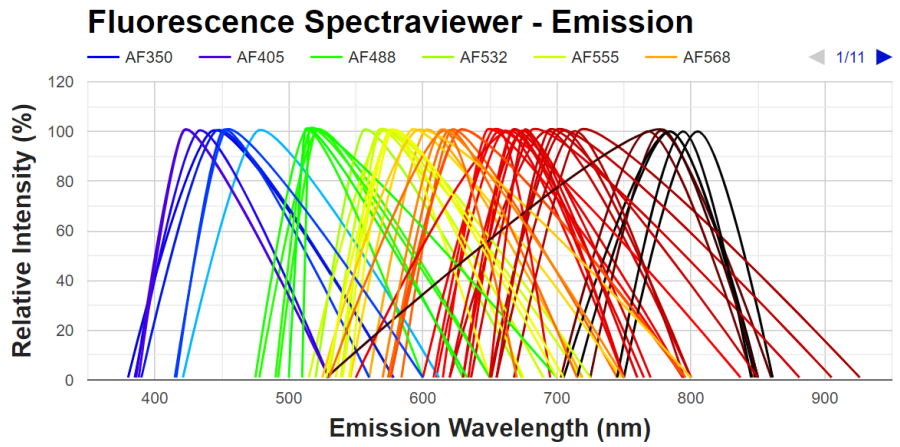
abbexa®

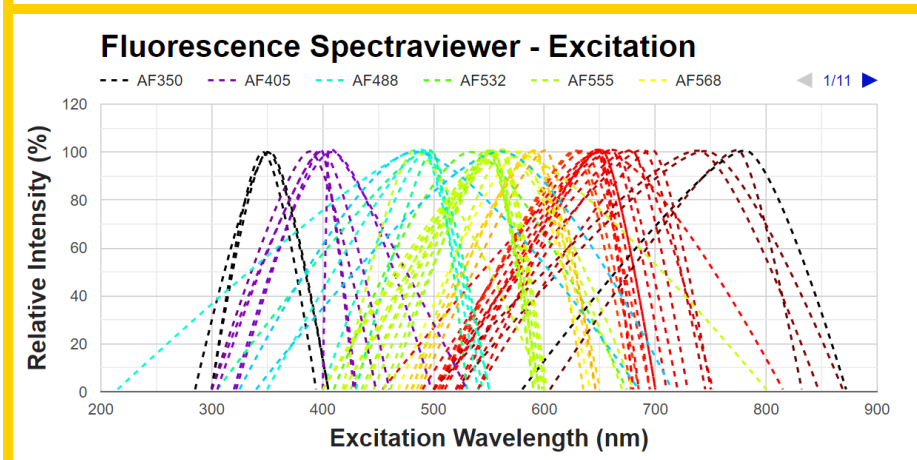
Abbexa also supplies a wide range of Secondary Antibodies for amplifying the signal of a primary antibody. These are provided for many target species, with a large variety of different labels for chemiluminescent, colorimetric and fluorescent detection. They are designed to accurately bind the target host's Primary Antibody Fc region for clearer and stronger detection. A Custom Production Service is available for the production and labelling of Secondary Antibodies.

Secondary Antibodies specifically recognise the Fc region of other antibodies. Exploitation of this interaction allows Secondary Antibodies to be used indirectly in the detection, purification and sorting of target antigens. For this, the Secondary Antibody must have specificity for the species and isotype of the Primary Antibody.

Therefore, when choosing a Secondary Antibody for detection, certain factors must be considered. The antibody chosen should be raised against the species of the Primary Antibody. For example, when using a mouse IgG monoclonal, the Secondary Antibody should be anti-mouse IgG.

The detection assay should also be considered. Western blotting and ELISAs permit the use of colorimetric, chemiluminescent and fluorescent conjugates. For immunofluorescence and flow cytometry, fluorescent reporters are generally the most applicable. All of our antibodies are supplemented with a comprehensive datasheet including tested applications.





Secondary Antibodies

abbexa®

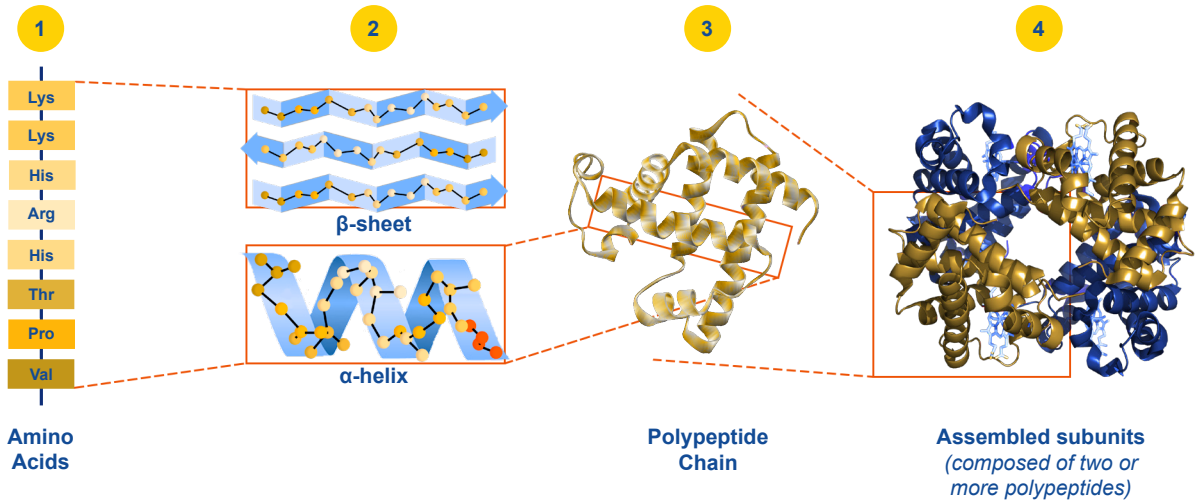
Peptides are short chains of amino acid monomers linked by amide bonds, usually between 2 to 50 amino acids in length. They are distinguished from proteins by their smaller size. Proteins are longer amino acid chains that may be bound to other chains as well as prosthetic groups. They are essential to organisms and participate in the vast majority of cellular processes.

An extensive range of proteins and peptides validated in numerous applications including SDS-PAGE, Western blotting, ELISA and immunoprecipitation are available from Abbexa.

Proteins for use as positive controls and standards are available to ensure quality and reliability of assays along with proteins such as GFP and Streptavidin-HRP for detection.

Peptides are most commonly used as immunogens to produce antibodies to a very specific region of a protein, eliminating the need to purify the protein of interest. When non-specific binding is an issue, for example in Western blotting and IHC, peptides can be used as blockers to block the antibody's binding sites. Proteins and peptides can also be used as inhibitors and for the study of protein structure and function.

Protein Structure



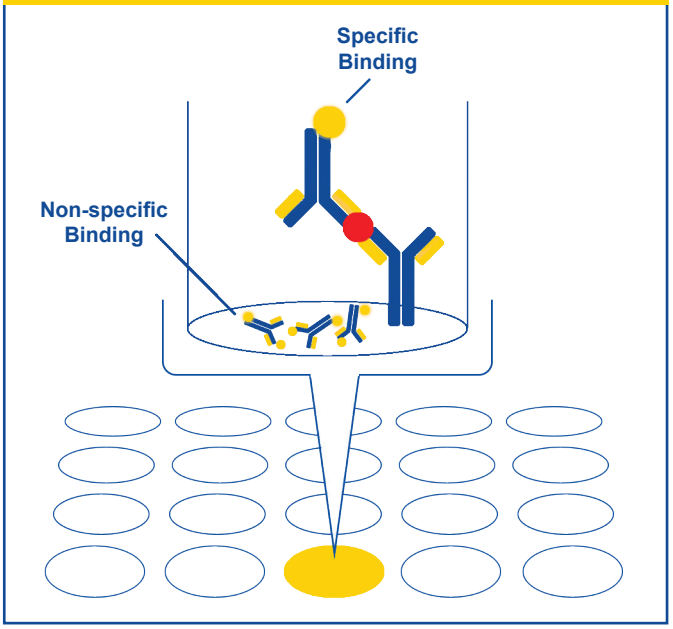
Proteins and Peptides

abbexa®

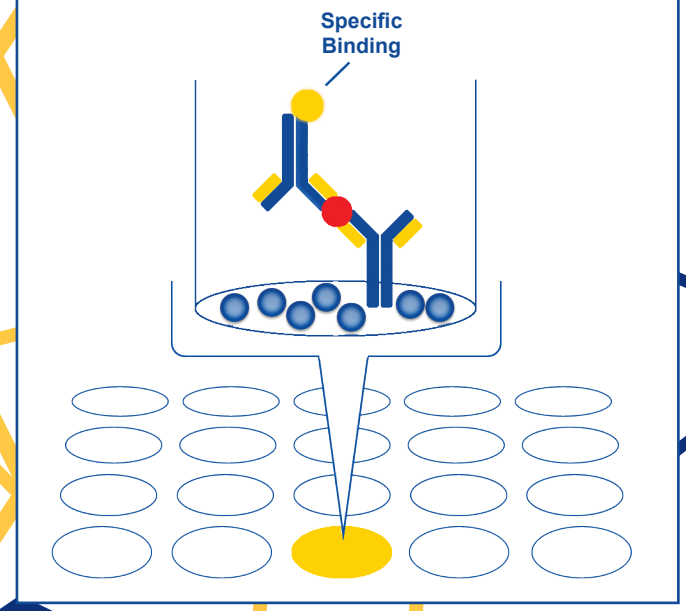
Blocking reagents are used to prevent non- specific binding within the assay. Proteins and other biomolecules may bind to unoccupied spaces on the surface of the wells. This can be detrimental to the specificity and sensitivity of the assay results, as less of the target antigen will be able to bind.

For example, occasionally multiple bands will be observed in Western Blotting when probing with a primary antibody. To eliminate this, a blocking buffer should be used, usually 5% BSA or non-fat dried milk. Non-specific binding is more prevalent with polyclonal antibodies but can also occur with monoclonal antibodies.

Without Blocking Buffer



With Blocking Buffer



Two major classes of blocking reagents are proteins and detergents.

BSA is a common type of protein blocker which will block the unoccupied spaces on the target surface. It will also help to reduce denaturation.

More information about the detergents available can be found in the Tools section.

The surface to be blocked dictates the type of blocker that should be chosen.

ELISA Kits

abbexa®

Enzyme-linked Immunosorbent Assay (ELISA) Kits are used to detect and quantify analytes. A reactant is immobilised to the assay plate either by direct adsorption or adsorption of a capture antibody. Once the desired analyte has been captured by the immobilised reactant, a detection antibody linked to an enzyme or other tag is added. This antibody is specific and will only bind to the analyte. The substrate is then introduced and the enzyme catalyses a reaction to produce a measurable result, e.g. a colour change.

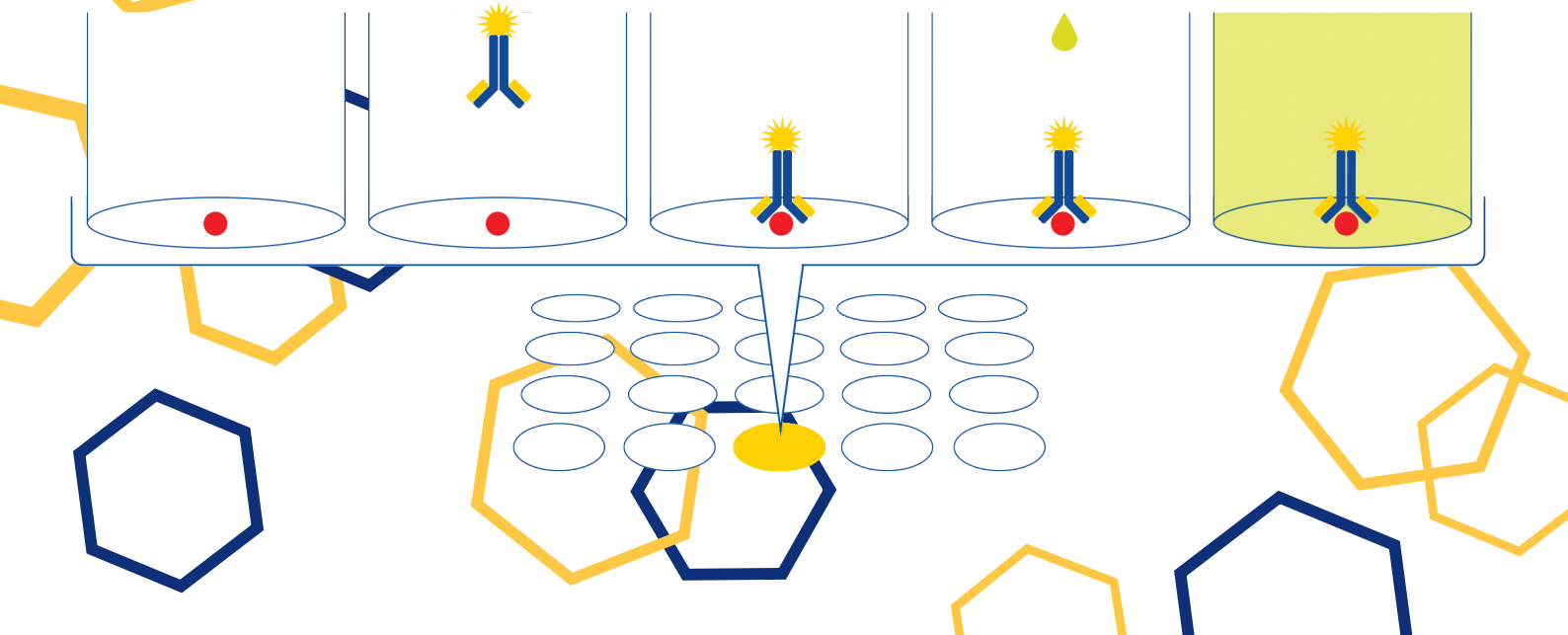
The four main types of ELISA Kits are: **Direct, Indirect, Sandwich and Competitive**

Direct ELISAs

Direct ELISAs are considered the simplest form of ELISA.

The sample is coated onto the plate either directly or via a capture antibody. Detection is via a labelled primary antibody which produces a colour change upon the addition of the substrate. Direct ELISAs are beneficial as no secondary antibody is required thus preventing cross reactivity between antibodies. This ELISA can also use a labelled antigen to detect an antibody coated onto the plate.

Compared to Indirect ELISAs, sensitivity is recognised to be lower due to the signal being less amplified. However, they can be performed much faster as only one step is required for detection.



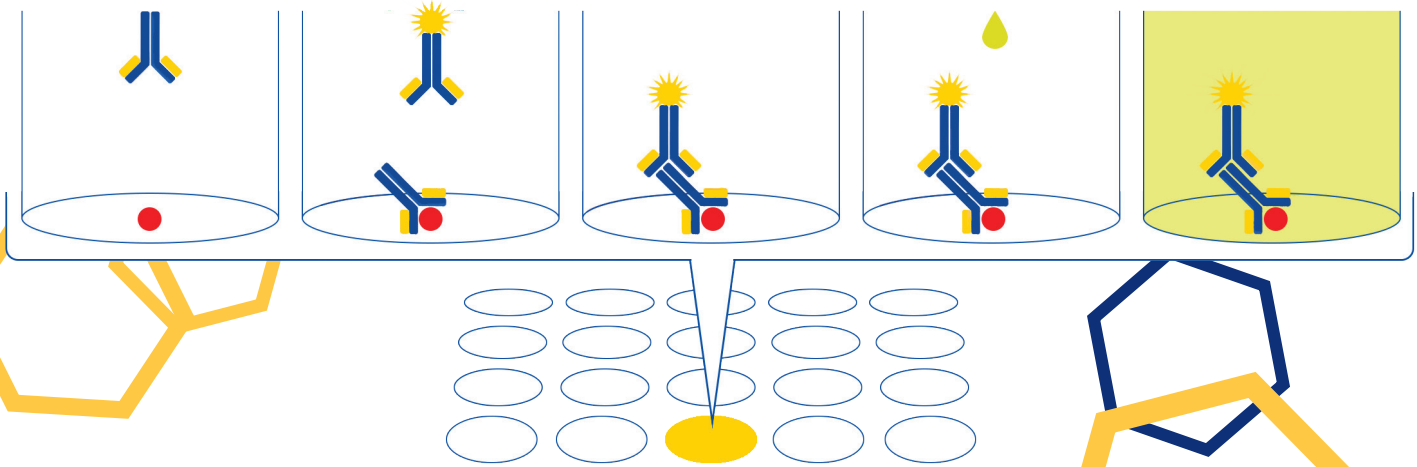
ELISA Kits

abbexa®

Indirect ELISAs

Indirect ELISAs use a secondary antibody conjugated to an enzyme to detect the primary antibody. The analyte is first coated onto the plate before primary antibody is added which will specifically bind to the analyte. Labelled secondary antibody is then introduced which will bind the primary antibody at a different epitope to the analyte. The addition of substrate results in a colour change, the intensity of which correlates with the concentration of analyte.

This type of ELISA has increased sensitivity due to the presence of multiple epitopes on the primary antibody that can be bound by the labelled secondary antibody. Flexibility may also be increased as more than one secondary detection antibody can be used with a single primary detection antibody. There may also be a reduction in cost to perform this assay as only one type of antibody will need to be labelled.

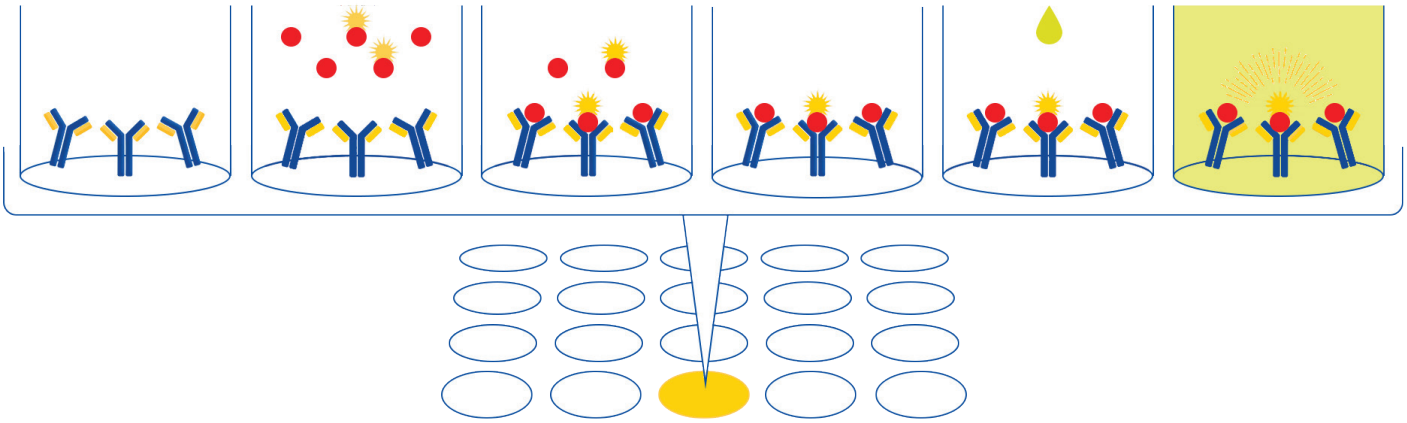


Competitive ELISAs

Competitive ELISA's are commonly used to determine small molecules such as lipids, hormones and small peptides. A purified antigen is labelled and competes with the unlabelled antigen in the sample to bind the capture antibody. Signal output is measured and compared with an expected signal output to determine the concentration of analyte present in the sample.

ELISA Kits

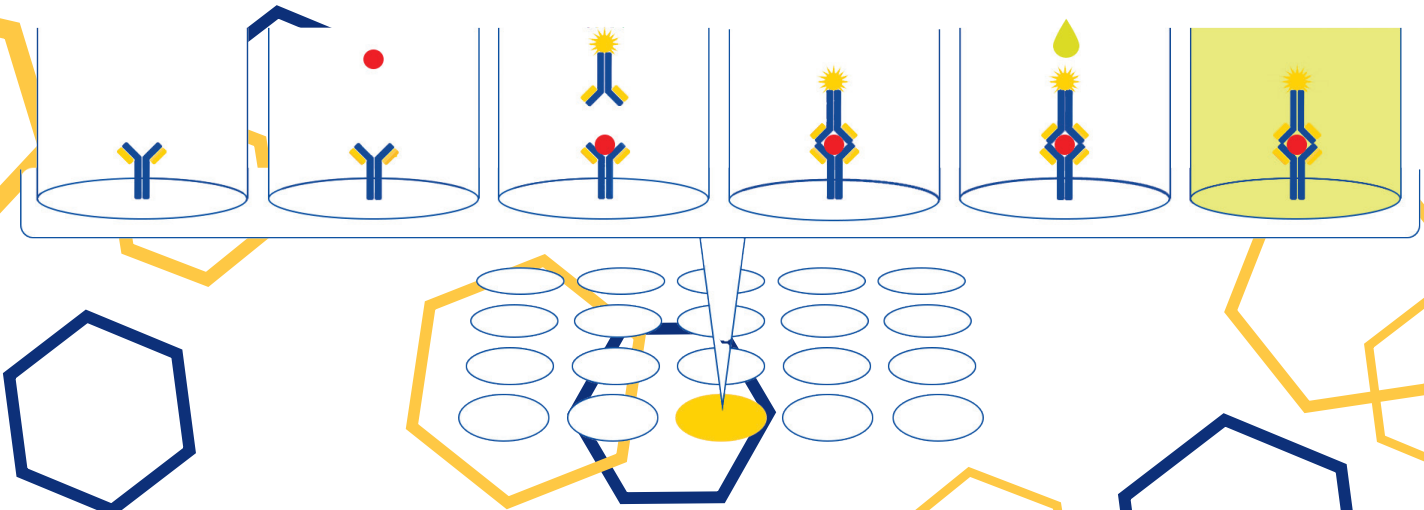
abbexa®



Sandwich ELISAs

Sandwich ELISAs tend to be the most readily recognised type of ELISA. The analyte to be measured is sandwiched, as the name suggests, between two antibodies, one for capture and one for detection. Detection can either be directly or indirectly. The analyte for Sandwich ELISA's should be fairly large in size to ensure the antibodies used are able to bind at different epitopes. When carrying out a Sandwich ELISA it is important that the antibodies used are matched pairs. Matched pairs refers to antibodies being specifically tested together to ensure that they bind to different epitopes of an antigen. This prevents the chance of the antibodies binding to the same site or recognising each other.

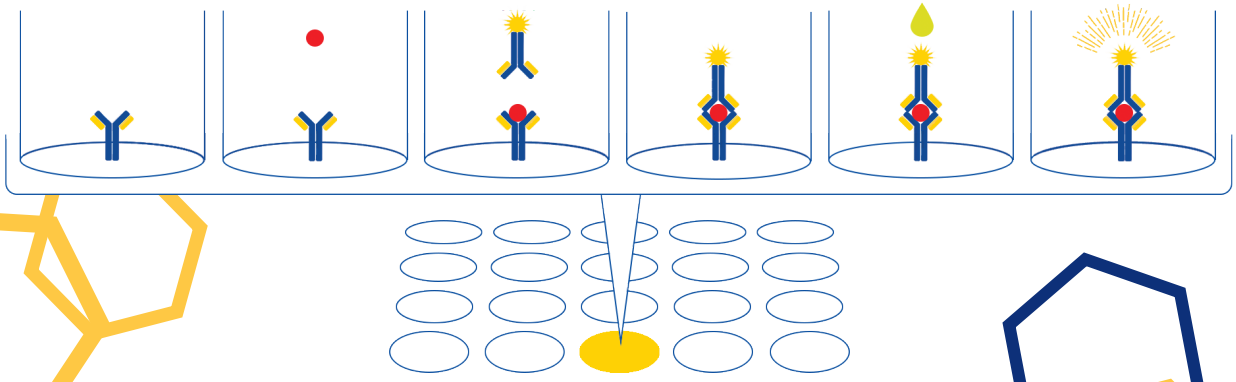
This technique is beneficial when the target analyte concentration is low. Following binding to the capture antibody, any other proteins within the sample that have not bound will be washed away.



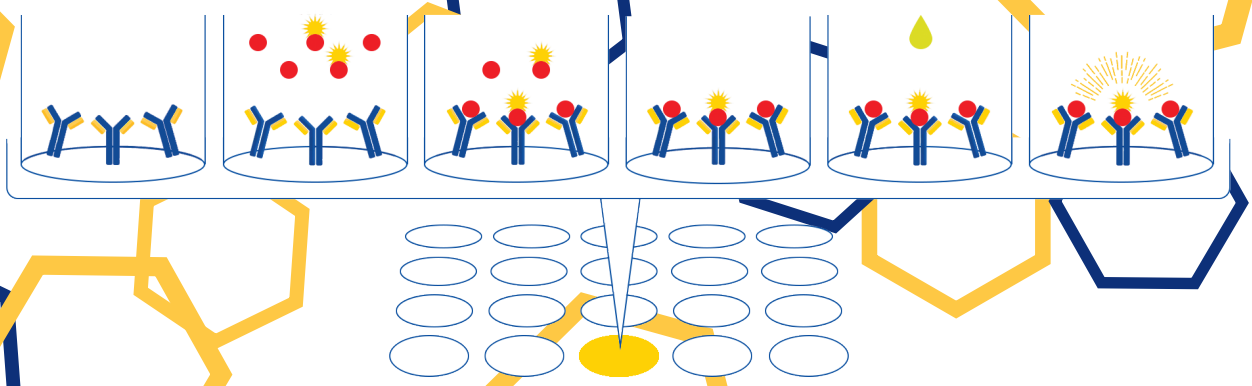
CLIA Kits

Chemiluminescence Immunoassay (CLIA) Kits are a variation of the standard ELISA. They are highly sensitive and possess a wide dynamic range. Instead of producing a colour change, an oxidation reaction excites a chemiluminescent substance forming an excited intermediate. Upon the return to ground state of the excited intermediate, a photon is released, which can be detected by a luminometer. An enhancer can be used which acts as an enzyme protector to enhance the chemiluminescent reaction by allowing the reaction to occur for a longer period of time without a reduction in the light output. CLIA Kits have a superior lower end sensitivity due to the signal multiplication and amplification. Abbexa offers **Sandwich and Competitive CLIA kits.**

Sandwich CLIAs



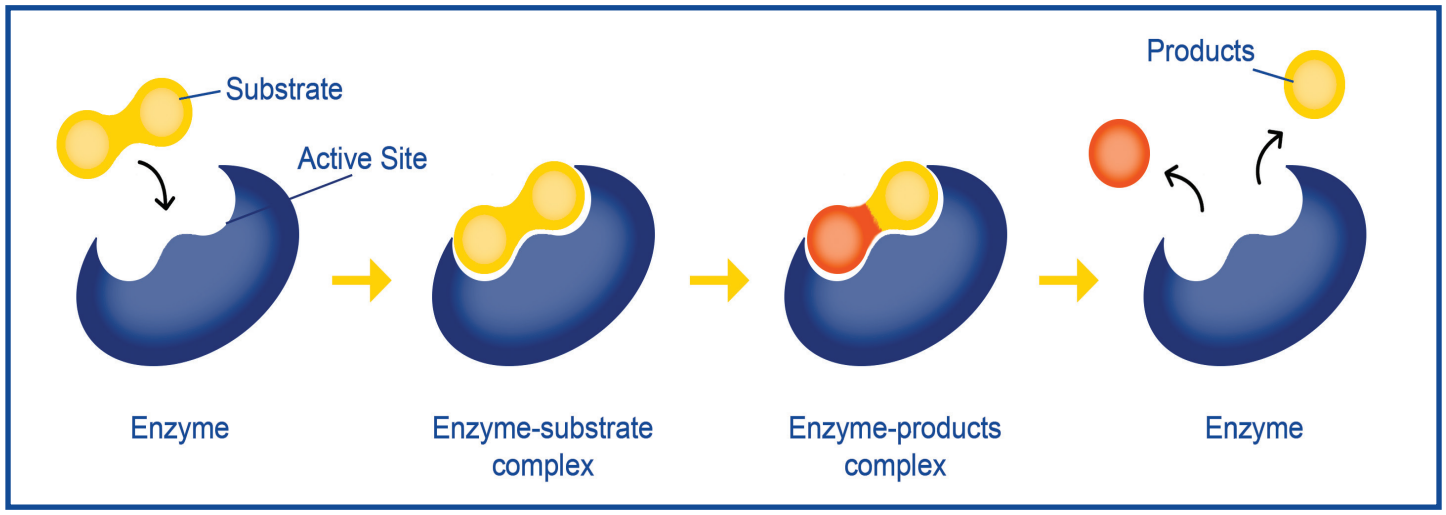
Competitive CLIAs



Enzymes

abbexa®

Enzymes are biological catalysts that speed up reactions by providing an alternative reaction pathway of lower activation energy. Their unique, three-dimensional structure dictates the specificity, and unlike chemical catalysts, enzymes are highly selective and will only catalyse certain reactions. Each enzyme has one or more active sites which are specific in shape to the target substrate. Enzymes work at optimum temperatures and pH levels to catalyse a reaction. When exposed to unfavourable temperatures or chemical denaturants, the 3D structure is disrupted and activity is lost. Enzymes do not undergo permanent changes and do not become part of the final product they catalyse, so they are able to continue reacting until all substrate available has been depleted.



A variety of enzymes encompassing all classes are available for numerous applications:

1. The oxidoreductases such as dehydrogenases and oxidases catalyse oxidation/reduction reactions via the transfer of hydrogen and oxygen atoms or electrons from one substance to another.

2. Functional groups such as methyl-, acyl-, amino- and phosphate groups are transferred between substances using the transferases, for example transaminase and kinase.

3. Enzymes which catalyse the hydrolytic cleavage of bonds including C-O, C-N and C-C belong to the hydrolases group. This consists of enzymes such as lipase, amylase and peptidase.

4. Another group responsible for cleavage reactions are the lyases,which cleave bonds such as C-C, C-O and C-N by elimination resulting in double bonds or rings or addition of groups.

5. Intramolecular rearrangements, geometric and structural, such as isomerisation are catalysed by the isomerases. They may also be called racemases, epimerases, cis-trans-isomerases, tautomerases, mutases and cycloisomerases. The subclasses reflect the type of isomerism and the sub-subclasses indicate the type of substrates.

Custom Antibodies

abbexa®

Abbexa also offers a professional Custom Antibody Production Service of rabbit polyclonal and mouse monoclonal antibodies.

This is a comprehensive service which covers immunogen design and production, immunisation, purification and validation.

The team provides support throughout the whole production process.

Peptide design and synthesis or full-length protein production

Protein antigens can be naturally occurring or recombinant. If there is an epitope of interest on the protein, its suitability will be analysed. If it is not suitable, a secondary epitope of interest can be selected or one can be suggested by the team. The size of the peptide and its propensity to aggregate can both affect the quality of the antibody produced. In general, the larger the peptide, the better the peptide will be at eliciting an immune response. Once the peptide has been finalised, it will be produced it and coupled to KLH (keyhole limpet hemocyanin) ready for immunisation. The full-length protein can also be used as an immunogen.

1

Antibody production and selection

A 5-round immunisation process ensures optimal results. The antibody is produced in a highly regulated clean room to ensure superior quality. The clonality chosen, monoclonal or polyclonal, will dictate the method for immunisation. Monoclonal Antibodies undergo a thorough selection process in which the set of cells producing the best quality antibody in the highest quantites is selected. If the antibody is intended for a particular application, the selected antibody will be screened for this.

2

Distributed by:

CliniSciences Group

Custom Antibodies

abbexa®

Antibody Purification

3

This step involves separating the antibody from other proteins. Purification is required to prevent undesired interactions during assay procedures. Three methods of purification are available: IgG/IgA purification, immunogen column purification and ammonium sulphate precipitation purification.

The team can provide advise regarding the suitability of each method, but the final decision will depend on which is most applicable to the antibody's final use. Ammonium sulphate precipitation is commonly used as a primary step in purification. Antibodies will precipitate at a lower concentration of ammonium sulphate than most components in serum therefore it is good for the concentration and enrichment of the antibody. IgG/IgA purification will purify all antibodies of the target class which is immobilised onto the solid phase. Immunogen column purification uses the antigen to bind the antibody, all other components of the solution are discarded, including non-specific immunoglobulins.

Antibody testing

In addition to the standard testing of custom antibodies Abbexa offers an additional array of popular screening and testing methods to validate use in specific applications. Testing is offered for: Enzyme-Linked Immunosorbent Assay (ELISA), Western Blot (WB), Immunohistochemistry (IHC) and Immunoprecipitation (IP).

Peptide design, protein production and antibody testing are also offered as independent services.

4

CliniSciences Group

Austria
Company: CliniSciences GmbH
Address: Sternwartestrasse 76, A-1180 Wien - Austria
Telephone: +43 720 115 580
Fax: +43 720 115 577
Email: oesterreich@clinisciences.com
Web: https://www.clinisciences.com

Belgium
Company: CliniSciences S.R.L
Address: Avenue Stalingrad 52, 1000 Brussels - Belgium
Telephone: +32 2 31 50 800
Fax: +32 2 31 50 801
Email: belgium@clinisciences.com
Web: https://www.clinisciences.com

Denmark
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: danmark@clinisciences.com
Web: https://www.clinisciences.com

Finland
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: suomi@clinisciences.com
Web: https://www.clinisciences.com

France
Company: CliniSciences S.A.S
Address: 74 Rue des Suisses, 92000 Nanterre- France
Telephone: +33 9 77 40 09 09
Fax: +33 9 77 40 10 11
Email: info@clinisciences.com
Web: https://www.clinisciences.com

Germany
Company: Biotrend Chemikalien GmbH
Address: Wilhelm-Mauser-Str. 41-43, 50827 Köln - Germany
Telephone: +49 221 9498 320
Fax: +49 221 9498 325
Email: info@biotrend.com
Web: https://www.biotrend.com

Iceland
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: island@clinisciences.com
Web: https://www.clinisciences.com

Ireland
Company: CliniSciences Limited
Address: Ground Floor, 71 lower Baggot street Dublin D02 P593 - Ireland
Telephone: +353 1 6971 146
Fax: +353 1 6971 147
Email: ireland@clinisciences.com
Web: https://www.clinisciences.com

Italy
Company: CliniSciences S.r.l
Address: Via Maremmana inferiore 378 Roma 00012 Guidonia Montecelio - Italy
Telephone: +39 06 94 80 56 71
Fax: +39 06 94 80 00 21
Email: italia@clinisciences.com
Web: https://www.clinisciences.com

Netherlands
Company: CliniSciences B.V.
Address: Kraijenhoffstraat 137A, 1018RG Amsterdam, Netherlands
Telephone: +31 85 2082 351
Fax: +31 85 2082 353
Email: nederland@clinisciences.com
Web: https://www.clinisciences.com

Norway
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: norge@clinisciences.com
Web: https://www.clinisciences.com

Poland
Company: CliniSciences sp.Z.o.o.
Address: ul. Rotmistrza Witolda Pileckiego 67 lok. 200 - 02-781 Warszawa -Poland
Telephone: +48 22 307 0535
Fax: +48 22 307 0532
Email: polska@clinisciences.com
Web: https://www.clinisciences.com

Portugal
Company: Quimigen Unipessoal LDA
Address: Rua Almada Negreiros, Lote 5, Loja 14, 2615-275 Alverca Do Ribatejo - Portugal
Telephone: +351 30 8808 050
Fax: +351 30 8808 052
Email: info@quimigen.com
Web: https://www.quimigen.pt

Spain
Company: CliniSciences Lab Solutions
Address: C/ Hermanos del Moral 13 (Bajo E), 28019, Madrid - Spain
Telephone: +34 91 269 40 65
Fax: +34 91 269 40 74
Email: espana@clinisciences.com
Web: https://www.clinisciences.com

Sweden
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: sverige@clinisciences.com
Web: https://www.clinisciences.com

Switzerland
Company: CliniSciences Limited
Address: Marktgasse 18 8302 Kloten - Switzerland
Telephone: +41 (044) 805 76 81
Fax: +41 (044) 805 76 75
Email: switzerland@clinisciences.com
Web: https://www.clinisciences.com

UK
Company: CliniSciences Limited
Address: 11 Progress Business center, Whittle Parkway, SL1 6DQ Slough- United Kingdom
Telephone: +44 (0)1753 866 511
or +44 (0) 330 684 0982
Fax: +44 (0)1753 208 899
Email: uk@clinisciences.com
lWeb: https://www.clinisciences.com

USA
Company: Biotrend Chemicals LLC
Address: c/o Carr Riggs Ingram, 500 Grand Boulevard, Suite 210 Miramar Beach, FL 32550- USA
Telephone: +1 850 650 7790
Fax: +1 850 650 4383
Email: info@biotrend-usa.com
Web: https://www.biotrend-usa.com