Masterclass Certificate in ELISA Assays

ELISA Troubleshooting Strategies

Absorbance refers to the amount of light absorbed by a solution, which is a critical measure in ELISA assays, where it is used to quantify the amount of antigen or antibody present, with higher absorbance values typically indicating higher concentrations of the molecule of interest, and optical density is often used interchangeably with absorbance.
Related terms include optical density, spectrophotometry, and microplate reader.
Absorbance is measured using a spectrophotometer or microplate reader, and the results are often expressed as optical density (OD) values, which can be used to calculate the concentration of the molecule of interest.
For example, in an ELISA assay, the absorbance of a sample is measured at a specific wavelength, typically 450 nm, and the resulting OD value is used to determine the concentration of the antigen or antibody present.

Antibody refers to a protein produced by the immune system in response to the presence of a foreign substance, such as a virus or bacteria, and is used in ELISA assays to detect and quantify specific molecules, with different types of antibodies, including monoclonal and polyclonal, each with its own unique characteristics and applications.
Related terms include antigen, epitope, and immunoglobulin.
Antibodies are composed of two heavy chains and two light chains, which are held together by disulfide bonds, and they bind to specific molecules, called antigens, through a region called the antigen-binding site.
For example, in an ELISA assay, a monoclonal antibody is used to detect the presence of a specific protein, and the antibody is conjugated to an enzyme, such as horseradish peroxidase, which catalyzes a colorimetric reaction, allowing the detection of the protein.

Antigen refers to a molecule that is recognized by the immune system as foreign and triggers an immune response, and is used in ELISA assays as the molecule being detected and quantified, with different types of antigens, including proteins, peptides, and carbohydrates, each with its own unique characteristics and applications.
Related terms include antibody, epitope, and immunogen.
Antigens are typically proteins or peptides, but can also be carbohydrates or other molecules, and they are recognized by the immune system through specific regions called epitopes.
For example, in an ELISA assay, a protein antigen is used as the molecule being detected, and a monoclonal antibody is used to bind to the antigen, allowing its detection and quantification.

Assay refers to a laboratory test or procedure used to detect and quantify specific molecules, such as proteins or antibodies, and is the core component of ELISA assays, where it is used to measure the concentration of a molecule of interest, with different types of assays, including direct, indirect, and sandwich, each with its own unique characteristics and applications.

Related terms include ELISA, microplate, and spectrophotometry.
Assays are typically performed in a microplate format, where multiple samples can be tested simultaneously, and the results are often expressed as optical density (OD) values, which can be used to calculate the concentration of the molecule of interest.
For example, in an ELISA assay, a sandwich assay is used to detect the presence of a specific protein, where a capture antibody is used to bind to the protein, and a detection antibody is used to bind to the capture antibody, allowing the detection and quantification of the protein.

Blocking refers to the process of preventing non-specific binding of molecules to a surface, such as a microplate or membrane, and is a critical step in ELISA assays, where it is used to reduce background noise and increase the specificity of the assay, with different types of blocking agents, including bovine serum albumin (BSA) and milk, each with its own unique characteristics and applications.
Related terms include non-specific binding, background noise, and signal-to-noise ratio.
Blocking is typically performed by incubating the surface with a blocking agent, such as BSA or milk, which binds to the surface and prevents other molecules from binding, reducing background noise and increasing the specificity of the assay.
For example, in an ELISA assay, a blocking agent is used to block non-specific binding to the microplate, allowing the specific binding of the antibody to the antigen, and increasing the signal-to-noise ratio of the assay.

Calibration refers to the process of adjusting the settings of a device, such as a microplate reader, to ensure accurate and consistent results, and is a critical step in ELISA assays, where it is used to ensure that the results are reliable and comparable, with different types of calibration methods, including single-point and multi-point calibration, each with its own unique characteristics and applications.
Related terms include validation, quality control, and standard curve.
Calibration is typically performed by using a set of standards with known concentrations, which are used to generate a standard curve, and the settings of the device are adjusted to ensure that the results are accurate and consistent.
For example, in an ELISA assay, a microplate reader is calibrated using a set of standards with known concentrations, allowing the accurate and consistent measurement of the absorbance of the samples.

Capture antibody refers to an antibody that is used to bind to a specific molecule, such as a protein or antigen, and is a critical component of ELISA assays, where it is used to capture the molecule of interest, allowing its detection and quantification, with different types of capture antibodies, including monoclonal and polyclonal, each with its own unique characteristics and applications.
Related terms include detection antibody, sandwich assay, and ELISA.
Capture antibodies are typically coated onto a surface, such as a microplate or membrane, and are used to bind to the molecule of interest, allowing its detection and quantification.
For example, in an ELISA assay, a capture antibody is used to bind to a specific protein, and a detection antibody is used to bind to the capture antibody, allowing the detection and quantification of the protein.

Conjugate refers to a molecule that is attached to an antibody or antigen, such as an enzyme or fluorophore, and is used in ELISA assays to detect and quantify specific molecules, with different types of

conjugates, including enzyme-linked and fluorophore-linked, each with its own unique characteristics and applications.
Related terms include enzyme-linked immunosorbent assay (ELISA), fluorophore, and biotin.
Conjugates are typically used to detect and quantify specific molecules, and are attached to antibodies or antigens through a variety of methods, including covalent bonding and affinity tagging.
For example, in an ELISA assay, a conjugate is used to detect the presence of a specific protein, where an enzyme-linked antibody is used to bind to the protein, and the enzyme catalyzes a colorimetric reaction, allowing the detection and quantification of the protein.

Control refers to a sample or standard that is used to validate the results of an assay, such as a positive or negative control, and is a critical component of ELISA assays, where it is used to ensure that the results are reliable and comparable, with different types of controls, including internal and external controls, each with its own unique characteristics and applications.
Related terms include validation, quality control, and standard curve.
Controls are typically used to validate the results of an assay, and are used to ensure that the results are reliable and comparable, with positive controls used to verify the presence of a molecule, and negative controls used to verify the absence of a molecule.
For example, in an ELISA assay, a positive control is used to verify the presence of a specific protein, and a negative control is used to verify the absence of the protein, allowing the validation of the results.

Detection antibody refers to an antibody that is used to detect the presence of a specific molecule, such as a protein or antigen, and is a critical component of ELISA assays, where it is used to bind to the molecule of interest, allowing its detection and quantification, with different types of detection antibodies, including monoclonal and polyclonal, each with its own unique characteristics and applications.
Related terms include capture antibody, sandwich assay, and ELISA.
Detection antibodies are typically conjugated to an enzyme or fluorophore, which is used to detect the presence of the molecule, and are used to bind to the molecule of interest, allowing its detection and quantification.
For example, in an ELISA assay, a detection antibody is used to bind to a specific protein, and an enzyme-linked conjugate is used to detect the presence of the protein, allowing the detection and quantification of the protein.

Direct assay refers to a type of assay where the molecule of interest is directly detected, without the use of a capture antibody, and is a common type of ELISA assay, where it is used to detect and quantify specific molecules, with different types of direct assays, including competitive and non-competitive, each with its own unique characteristics and applications.
Related terms include indirect assay, sandwich assay, and ELISA.
Direct assays are typically used to detect and quantify specific molecules, and are often used in situations where the molecule of interest is present in high concentrations, such as in serum or plasma samples.
For example, in an ELISA assay, a direct assay is used to detect the presence of a specific protein, where an enzyme-linked antibody is used to bind to the protein, and the enzyme catalyzes a colorimetric reaction, allowing the detection and quantification of the protein.

ELISA refers to a laboratory test or procedure used to detect and quantify specific molecules, such as proteins or antibodies, and is a common type of assay, where it is used to measure the concentration of a molecule of interest, with different types of ELISA assays, including direct, indirect, and sandwich, each with its own unique characteristics and applications.
Related terms include enzyme-linked immunosorbent assay, microplate, and spectrophotometry.
ELISA assays are typically performed in a microplate format, where multiple samples can be tested simultaneously, and the results are often expressed as optical density (OD) values, which can be used to calculate the concentration of the molecule of interest.
For example, in an ELISA assay, a sandwich ELISA is used to detect the presence of a specific protein, where a capture antibody is used to bind to the protein, and a detection antibody is used to bind to the capture antibody, allowing the detection and quantification of the protein.

Enzyme-linked immunosorbent assay (ELISA) refers to a laboratory test or procedure used to detect and quantify specific molecules, such as proteins or antibodies, and is a common type of assay, where it is used to measure the concentration of a molecule of interest, with different types of ELISA assays, including direct, indirect, and sandwich, each with its own unique characteristics and applications.
Related terms include microplate, spectrophotometry, and antibody.
ELISA assays are typically performed in a microplate format, where multiple samples can be tested simultaneously, and the results are often expressed as optical density (OD) values, which can be used to calculate the concentration of the molecule of interest.
For example, in an ELISA assay, a sandwich ELISA is used to detect the presence of a specific protein, where a capture antibody is used to bind to the protein, and a detection antibody is used to bind to the capture antibody, allowing the detection and quantification of the protein.

Epitope refers to a region on a molecule that is recognized by the immune system, and is a critical component of ELISA assays, where it is used to bind to antibodies, allowing the detection and quantification of specific molecules, with different types of epitopes, including linear and conformational, each with its own unique characteristics and applications.
Related terms include antigen, antibody, and immunogen.
Epitopes are typically composed of a specific sequence of amino acids, and are recognized by the immune system through specific interactions with antibodies, allowing the detection and quantification of specific molecules.
For example, in an ELISA assay, an epitope is used to bind to a specific antibody, allowing the detection and quantification of a specific protein.

Fluorophore refers to a molecule that emits light at a specific wavelength, and is used in ELISA assays to detect and quantify specific molecules, with different types of fluorophores, including fluorescent dyes and proteins, each with its own unique characteristics and applications.
Related terms include fluorescence, biotin, and conjugate.
Fluorophores are typically used to detect and quantify specific molecules, and are attached to antibodies or antigens through a variety of methods, including covalent bonding and affinity tagging.
For example, in an ELISA assay, a fluorophore is used to detect the presence of a specific protein, where a fluorescent dye is used to bind to the protein, and the fluorescence is measured, allowing the detection and

quantification of the protein.

Horseradish peroxidase (HRP) refers to an enzyme that is commonly used in ELISA assays to catalyze a colorimetric reaction, and is a critical component of ELISA assays, where it is used to detect and quantify specific molecules, with different types of HRP conjugates, including enzyme-linked and fluorophore-linked, each with its own unique characteristics and applications.
Related terms include enzyme-linked immunosorbent assay (ELISA), microplate, and spectrophotometry.
HRP is typically used to catalyze a colorimetric reaction, where a substrate is converted into a colored product, allowing the detection and quantification of specific molecules.
For example, in an ELISA assay, HRP is used to detect the presence of a specific protein, where an enzyme-linked antibody is used to bind to the protein, and the HRP catalyzes a colorimetric reaction, allowing the detection and quantification of the protein.

Immune response refers to the body's defense against infection or disease, and is a critical component of ELISA assays, where it is used to detect and quantify specific molecules, such as antibodies or antigens, with different types of immune responses, including humoral and cell-mediated, each with its own unique characteristics and applications.
Related terms include antibody, antigen, and immunogen.
Immune responses are typically triggered by the presence of a foreign substance, such as a virus or bacteria, and involve the activation of immune cells, such as B cells and T cells, which produce antibodies and other molecules to fight the infection.
For example, in an ELISA assay, an immune response is used to detect the presence of a specific antibody, where a capture antibody is used to bind to the antibody, and a detection antibody is used to bind to the capture antibody, allowing the detection and quantification of the antibody.

Immunogen refers to a molecule that triggers an immune response, and is a critical component of ELISA assays, where it is used to detect and quantify specific molecules, such as antibodies or antigens, with different types of immunogens, including proteins, peptides, and carbohydrates, each with its own unique characteristics and applications.
Related terms include antigen, antibody, and epitope.
Immunogens are typically composed of a specific sequence of amino acids, and are recognized by the immune system through specific interactions with antibodies, allowing the detection and quantification of specific molecules.
For example, in an ELISA assay, an immunogen is used to detect the presence of a specific antibody, where a capture antibody is used to bind to the antibody, and a detection antibody is used to bind to the capture antibody, allowing the detection and quantification of the antibody.

Indirect assay refers to a type of assay where the molecule of interest is detected using a secondary antibody, and is a common type of ELISA assay, where it is used to detect and quantify specific molecules, with different types of indirect assays, including competitive and non-competitive, each with its own unique characteristics and applications.
Related terms include direct assay, sandwich assay, and ELISA.
Indirect assays are typically used to detect and quantify specific molecules, and are often used in situations

where the molecule of interest is present in low concentrations, such as in tissue or cell culture samples.
For example, in an ELISA assay, an indirect assay is used to detect the presence of a specific protein, where a primary antibody is used to bind to the protein, and a secondary antibody is used to bind to the primary antibody, allowing the detection and quantification of the protein.

Microplate refers to a small plate with multiple wells, used to perform multiple assays simultaneously, and is a critical component of ELISA assays, where it is used to detect and quantify specific molecules, with different types of microplates, including 96-well and 384-well, each with its own unique characteristics and applications.
Related terms include ELISA, spectrophotometry, and absorbance.
Microplates are typically made of plastic or other materials, and are designed to hold small volumes of liquid, such as 100-200 µL, allowing multiple assays to be performed simultaneously.
For example, in an ELISA assay, a microplate is used to detect the presence of a specific protein, where multiple samples are added to the wells, and the absorbance is measured, allowing the detection and quantification of the protein.

Non-specific binding refers to the binding of molecules to a surface or other molecules, without specificity, and is a critical component of ELISA assays, where it is used to reduce background noise and increase the specificity of the assay, with different types of non-specific binding, including hydrophobic and electrostatic, each with its own unique characteristics and applications.
Related terms include blocking, background noise, and signal-to-noise ratio.
Non-specific binding is typically reduced by using blocking agents, such as bovine serum albumin (BSA) or milk, which bind to the surface and prevent other molecules from binding, reducing background noise and increasing the specificity of the assay.
For example, in an ELISA assay, non-specific binding is reduced by using a blocking agent, allowing the specific binding of the antibody to the antigen, and increasing the signal-to-noise ratio of the assay.

Optical density (OD) refers to the measure of the amount of light absorbed by a solution, and is a critical component of ELISA assays, where it is used to quantify the amount of antigen or antibody present, with different types of OD measurements, including absorbance and transmittance, each with its own unique characteristics and applications.
Related terms include absorbance, spectrophotometry, and microplate reader.
OD is typically measured using a spectrophotometer or microplate reader, and the results are often expressed as OD values, which can be used to calculate the concentration of the molecule of interest.
For example, in an ELISA assay, the OD is measured at a specific wavelength, typically 450 nm, and the resulting OD value is used to determine the concentration of the antigen or antibody present.

Sandwich assay refers to a type of assay where the molecule of interest is detected using a capture antibody and a detection antibody, and is a common type of ELISA assay, where it is used to detect and quantify specific molecules, with different types of sandwich assays, including direct and indirect, each with its own unique characteristics and applications.
Related terms include direct assay, indirect assay, and ELISA.
Sandwich assays are typically used to detect and quantify specific molecules, and are often used in

situations where the molecule of interest is present in high concentrations, such as in serum or plasma samples.
For example, in an ELISA assay, a sandwich assay is used to detect the presence of a specific protein, where a capture antibody is used to bind to the protein, and a detection antibody is used to bind to the capture antibody, allowing the detection and quantification of the protein.

Spectrophotometry refers to the measurement of the interaction between light and molecules, and is a critical component of ELISA assays, where it is used to measure the absorbance of a solution, with different types of spectrophotometry, including UV-Vis and infrared, each with its own unique characteristics and applications.
Related terms include absorbance, optical density (OD), and microplate reader.
Spectrophotometry is typically used to measure the absorbance of a solution, and the results are often expressed as OD values, which can be used to calculate the concentration of the molecule of interest.
For example, in an ELISA assay, spectrophotometry is used to measure the absorbance of a solution, and the resulting OD value is used to determine the concentration of the antigen or antibody present.

Standard curve refers to a graphical representation of the relationship between the concentration of a molecule and the resulting signal, such as absorbance or fluorescence, and is a critical component of ELISA assays, where it is used to quantify the amount of antigen or antibody present, with different types of standard curves, including linear and non-linear, each with its own unique characteristics and applications.
Related terms include calibration, validation, and quality control.
Standard curves are typically generated by plotting the concentration of a molecule against the resulting signal, and are used to quantify the amount of antigen or antibody present in a sample.
For example, in an ELISA assay, a standard curve is used to quantify the amount of a specific protein, where the concentration of the protein is plotted against the resulting OD value, allowing the determination of the concentration of the protein in a sample.

Substrate refers to a molecule that is converted into a colored product by an enzyme, such as horseradish peroxidase (HRP), and is a critical component of ELISA assays, where it is used to detect and quantify specific molecules, with different types of substrates, including chromogenic and fluorogenic, each with its own unique characteristics and applications.
Related terms include enzyme-linked immunosorbent assay (ELISA), microplate, and spectrophotometry.
Substrates are typically used to detect and quantify specific molecules, and are converted into a colored product by an enzyme, allowing the detection and quantification of the molecule.
For example, in an ELISA assay, a substrate is used to detect the presence of a specific protein, where an enzyme-linked antibody is used to bind to the protein, and the substrate is converted into a colored product, allowing the detection and quantification of the protein.

Validation refers to the process of verifying the accuracy and reliability of an assay, and is a critical component of ELISA assays, where it is used to ensure that the results are reliable and comparable, with different types of validation methods, including internal and external validation, each with its own unique characteristics and applications.
Related terms include calibration, quality control, and standard curve.

Validation is typically performed by using a set of standards with known concentrations, which are used to generate a standard curve, and the results are compared to the expected values, allowing the verification of the accuracy and reliability of the assay.
For example, in an ELISA assay, validation is performed by using a set of standards with known concentrations, and the results are compared to the expected values, allowing the verification of the accuracy and reliability of the assay.