Agilent Dako

CE

General Instructions
Antibodies for use in ELISA

Purpose
These general instructions apply to Agilent Dako antibodies for use with enzyme linked immunosorbent assay (ELISA). Always consult the individual Instructions for Use of your specific product.

The General Instructions for Antibodies for use in ELISA are guidelines only. A protocol is provided in the specific Instructions for Use for each antibody product. Optimal procedures must be determined and verified by the user.

NOTE: Contact Technical Support via ReagentPartnership@agilent.com to report any unusual results. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the country in which the user and/or the patient is established.

Principle of Procedure
ELISA can be used for the quantitative detection of antigen in human test material through either direct detection, indirect detection, or a sandwich assay (see figure).

In direct and indirect ELISA assays, the antigen is typically coated on the bottom of a 96-well microplate suited for protein coupling.

In a direct ELISA, a primary Agilent Dako antibody conjugated to horseradish peroxidase (HRP) can be used for the detection of antigen coupled to the well.

In the more commonly used indirect ELISA, an unconjugated primary antibody with affinity for the target antigen is used in conjunction with a secondary Agilent Dako antibody reagent that is specific to the detection of the primary antibody. The secondary antibody is conjugated to HRP for the detection of the antigen coupled to the well.

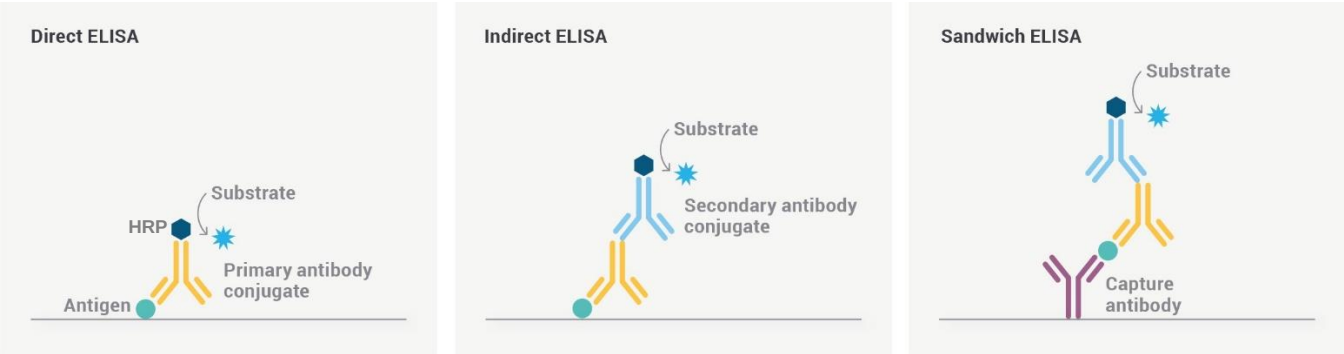
In a sandwich ELISA, the plate is first coated with an antigen-capturing antibody. Incubation with sample material leads to the capture of the target antigen by the antibody, while the remaining unbound sample is removed by washing. The antigen can then be detected using either a primary Agilent Dako antibody (direct sandwich ELISA) or a primary antibody reagent in conjunction with a secondary Agilent Dako antibody (indirect sandwich ELISA).

In all three types of ELISA assays, the final step is the addition of a 3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate. The HRP enzyme catalyzes a redox reaction of the TMB substrate causing a color change. The redox reaction is stopped by the addition of a stop solution, after which the color change can be quantified by reading the absorbance at 450 nm with 620 nm as a reference wavelength using an ELISA plate reader.

The generation of a standard curve using controls containing known levels of antigen allows for the quantitative determination of antigen in the sample material, based on the linear proportionality between antigen concentration and absorbance. The generated standard curve should be a linear relationship of the type:

$$Absorbance = K \times Concentration$$

Where K is the slope of the curve.



The user should read these General Instructions and product-specific Instructions for Use carefully and become familiar with all the components and the instrumentation prior to use.

Materials Required, but Not Supplied
See individual Instructions for Use for specific recommendation of procedure. Not all the below listed materials may be required.

Blood sampling tubes with anticoagulant

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Test tubes for sample preparation

Micropipettes with disposable tips

Positive control: The recommended control reagent should be a human control material value assigned for the individual target proteins.

Standards: The recommended standards should be a dilution range of a human control material value assigned for the individual target proteins.

Negative control: The recommended control reagent should be a human control material deficient in the individual target proteins.

Dilution and wash buffer: 0.01 M PBS, 0.5 M NaCl, 0,1% Tween 20, pH 7.2

TMB substrate, ready to use.

OPD (o-phenylenediamine dihydrochloride) tablets

Stop solution: 0.20 M sulphuric acid or as specified for the substrate

96-well microplate

ELISA plate reader

ELISA plate washer

Unconjugated antibody reagents for use as primary antibody in indirect and sandwich ELISA assays.

Storage

Store at 2–8 °C. The expiration date is stated on the vial and applies to both opened and unopened vials stored at 2–8 °C. Do not use after the expiration date.

If reagents are stored under any conditions other than those specified, the user must verify these conditions. There are no obvious signs to indicate instability of these products. Therefore, relevant controls should be run simultaneously with patient samples. If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact technical support via ReagentPartnership@agilent.com.

Specimen Preparation

These are guidelines only. Optimal procedures must be determined and verified by the user.

Serum

Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure that complete clot formation has taken place, which usually takes around 15–30 minutes. Separate serum from red blood cells after complete clot formation (after 30 minutes) but no later than 1 hour after blood draw.

EDTA-plasma

Use plasma collected by standard venipuncture techniques into glass or plastic tubes with EDTA. Ensure that centrifugation is adequate to remove platelets. Separate plasma from red blood cells no later than 1 hour after blood draw.

Ideally, blood samples should be processed within the specified time frame. When this is not possible, each laboratory should validate its collection and storage methods to maintain specimen integrity comparable to freshly processed material.

General Procedure

NOTE: Any modification of the recommendations outlined in the individual Instructions for Use must be validated by the user.

Direct ELISA

1. Use a precoated 96-well microplate or perform coating of an uncoated 96-well microplate according to manufacturer's guidelines.
2. Dilute the Agilent Dako antibody conjugate according to specifications provided in the antibody's Instructions for Use.
3. Add 100 µL diluted antibody conjugate to wells.
4. Incubate 1 hour at room temperature (20–25 °C) on a shaker at 500 rpm.
5. Wash three times using wash buffer. It is recommended to use an ELISA plate washer.

   If manual wash is performed, follow these instructions:

   a. Discard all liquid in the wells
   b. Fill all wells completely with wash buffer
   c. Discard the wash buffer
   d. Ensure complete evacuation of wash buffer by lightly tapping the emptied plate on a piece of absorbent paper.
   e. Perform steps b-d three times
6. Add 100 µL TMB substrate to each well. Time of development is typically 5–30 minutes. It is recommended to keep the plate in the dark during development.
7. Add 100 µL stop solution to each well, following the same order as for addition of TMB substrate to avoid variations in incubation time.
8. Read absorbance at 450 nm with 620 nm as a reference wavelength using an ELISA plate reader.

Indirect ELISA

1. Use a precoated 96-well microplate or perform coating of an uncoated 96-well microplate according to manufacturer's guidelines.
2. Dilute the primary antibody reagent according to manufacturer's specification or internally validated protocol.
3. Add 100 µL diluted primary antibody reagent to wells.
4. Incubate 1 hour at room temperature on a shaker at 500 rpm.
5. Wash three times using wash buffer. It is recommended to use an ELISA plate washer.
   If manual wash is performed, follow these instructions:
   a. Discard all liquid in the wells
   b. Fill all wells completely with wash buffer
   c. Discard the wash buffer
   d. Ensure complete evacuation of wash buffer by lightly tapping the emptied plate on a piece of absorbent paper.
   e. Perform steps b-d three times
6. Add 100 µL Agilent Dako antibody conjugate, diluted per specifications in the specific product Instructions for Use, to each well.
7. Incubate 1 hour at room temperature on a shaker at 500 rpm.
8. Wash three times using wash buffer. It is recommended to use an ELISA plate washer.
9. Add 100 µL TMB substrate to each well. Time of development is typically 5–30 minutes. It is recommended to keep the plate in the dark during development.
10. Add 100 µL stop solution to each well, following the same order as for addition of TMB substrate to avoid variations in incubation time.
11. Read absorbance at 450 nm with 620 nm as a reference wavelength using an ELISA plate reader.

Sandwich ELISA

1. Use an antibody-precoated 96-well microplate or perform coating of an uncoated 96-well microplate according to manufacturer's guidelines.
2. Prepare standards and sample dilutions in dilution buffer according to manufacturer's specification or internally validated protocol.
3. Add 100 µL of standards and samples to the plate.
4. Incubate 1 hour at room temperature on a shaker at 500 rpm.
5. Wash three times using wash buffer. It is recommended to use an ELISA plate washer.
6. Dilute the primary antibody reagent according to manufacturer's specification or internally validated protocol.
7. Add 100 µL diluted primary antibody reagent to wells.
8. Incubate 1 hour at room temperature on a shaker at 500 rpm.
9. Wash three times using wash buffer. It is recommended to use an ELISA plate washer.
10. Add 100 µL Agilent Dako antibody conjugate, diluted per specifications in the specific product Instructions for Use, to each well.
11. Incubate 1 hour at room temperature on a shaker at 500 rpm.
12. Wash three times using wash buffer. It is recommended to use an ELISA plate washer.
    If manual wash is performed, follow these instructions:
    a. Discard all liquid in the wells
    b. Fill all wells completely with wash buffer
    c. Discard the wash buffer
    d. Ensure complete evacuation of wash buffer by lightly tapping the emptied plate on a piece of absorbent paper.
    e. Perform steps b-d three times
13. Add 100 µL TMB substrate to each well. Time of development is typically 5–30 minutes. It is recommended to keep the plate in the dark during development.
14. Add 100 µL stop solution to each well, following the same order as for addition of TMB substrate to avoid variations in incubation time.
15. Read absorbance at 450 nm with 620 nm as a reference wavelength using an ELISA plate reader.

Quality Control

Deviations in the recommended procedures for sample preparation and processing in the user's laboratory may produce significant variability in results. Therefore, each plate should include a positive and a negative control sample to ensure validity of the results.

It is recommended to run all samples in duplicates.

Troubleshooting

In case of unexpected results, ensure that all steps in the procedure were performed as outlined in the specific Instructions for Use and general Instructions. The TMB should be colorless when added to the plate and should not be exposed to light until use, and the plate should not be allowed to dry out at any point during testing. Poor washing or cross reactivity with other animal species may lead to increased background signals, which can lead to data loss. Refer to the specific Instructions for Use for your product for information on cross reactivity. If the issue persists, please contact technical support via **ReagentPartnership@agilent.com**.

Interfering Substances

Grease smears, fingerprints, or other contaminants on the bottom of the microplate may cause elevated background signal. It is therefore recommended to avoid touching the bottom of the microplate until after the final measurement.

Some analytes may have specific interferences. Refer to the specific antibody detecting the analyte for interfering substances.

General Limitations

1. ELISA assays are dependent on the handling and processing of the samples prior to testing. Improper handling, freezing, thawing, heating, or contamination with other tissues or fluids may cause false-negative or false-positive results.
2. The clinical interpretation of any results must be made in the context of the patient's clinical history by a certified professional.
3. Unless specifically claimed in the instructions, the performance characteristics of antibodies used for ELISA techniques have not been determined for other laboratory techniques.
4. When properly stored, the reagent is stable to the date indicated on the label. Some degree of enzyme activity loss shall be expected over the products shelf life. Do not use reagent beyond the expiration date. Improper storage and use of reagents may lead to erroneous results.

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Revision [01]
Date of issue: 2022.03

Changes since last revision	
Revision [01]	Creation