1. DESCRIPTION AND PRINCIPLE

How do our ELISA kits work?

The ELISA Genie ELISA (enzyme-linked immunosorbent assays) assay kits are designed for the measurement of analytes in a wide variety of samples. As today’s scientists demand high quality consistent data, we have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands.

This ELISA Genie kit uses a Competitive-ELISA technique. The microtiter plate provided in this kit has been pre-coated with the analyte. During the reaction, the analyte in the sample or standard competes with a fixed amount of analyte on the solid phase support for sites on the Biotinylated Detection Antibody specific to the analyte. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of the analyte in the sample is then determined by comparing the O.D. of the samples to the standard curve.
2. KIT CONTENTS

Each kit contains reagents for 48 or 96 assays including:

<table>
<thead>
<tr>
<th>No.</th>
<th>Component</th>
<th>48-Well Kit</th>
<th>96-Well Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>1 vial</td>
<td>2 vials</td>
</tr>
<tr>
<td>2</td>
<td>Sample/Standard Dilution buffer</td>
<td>10ml</td>
<td>20ml</td>
</tr>
<tr>
<td>3</td>
<td>ELISA Strip plate</td>
<td>6 strips x 8 wells</td>
<td>12 strips x 8 wells</td>
</tr>
<tr>
<td>4</td>
<td>Wash Buffer (25x)</td>
<td>15ml</td>
<td>30ml</td>
</tr>
<tr>
<td>5</td>
<td>SABC dilution buffer</td>
<td>5ml</td>
<td>10ml</td>
</tr>
<tr>
<td>6</td>
<td>Antibody dilution buffer</td>
<td>5ml</td>
<td>10ml</td>
</tr>
<tr>
<td>7</td>
<td>HRP-Streptavidin Conjugate (SABC)</td>
<td>60µl</td>
<td>120µl</td>
</tr>
<tr>
<td>8</td>
<td>Biotin-detection antibody (Concentrated)</td>
<td>30µl</td>
<td>60µl</td>
</tr>
<tr>
<td>9</td>
<td>Stop Solution</td>
<td>5ml</td>
<td>10ml</td>
</tr>
<tr>
<td>10</td>
<td>TMB Substrate</td>
<td>5ml</td>
<td>10ml</td>
</tr>
<tr>
<td>11</td>
<td>Plate Sealer</td>
<td>3 pieces</td>
<td>5 pieces</td>
</tr>
<tr>
<td>12</td>
<td>Manual</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Additional materials required:

1. 37°C incubator
2. Plate shaker
3. Plate Reader with 450nm filter
4. Precision pipettes and disposable pipette tips
5. Distilled water
6. Disposable tubes for sample dilution
7. Absorbent paper
3. WORKFLOW OVERVIEW

- Prepare plate standards and reagents
- Wash plate x2
- Add 50µl of standard blank and sample
- Immediately add 50µl Biotin labelled antibody working solution
- Wash plate x3
- Add 100µl SABC working solution
- Wash plate x5
- Add 90µl TMB Substrate
- Add 50µl STOP solution and read absorbance at 450nm
4. SHIPPING AND STORAGE

ELISA Genie ELISA kits are shipped in ice packs. Please store your kit at 4°C for 6 months.

LOOKING FOR TECHNICAL SUPPORT?

Email hello@elisagenie.com with any queries.
**General considerations:** According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C) and for optimal results, avoid repeated freeze-thaw cycles.

**Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 mins before centrifugation for 15 mins at 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.

**Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Note: over hemolyzed samples are not suitable for use with this kit.

**Urine & Cerebrospinal fluid:** Collect in a sterile container, centrifuge for 20 mins at 2000-3000 rpm. Remove supernatant and if any precipitation is detected, repeat the centrifugation step. A similar protocol can be used for cerebrospinal fluid.

**Cell culture supernatant:** Collect supernatant and centrifuge at 4°C for 20 mins at 2000-3000 rpm. Remove supernatant and rinse cells 2 times with PBS (pH 7.2-7.4) and perform a total cell count. Optimal cell concentration is 1 million/ml. To release cellular components, dilute the cell pellet in PBS and use 3-4 freeze-thaw cycles in liquid Nitrogen (commercial lysis buffers can be used according to manufacturer’s instructions). Centrifuge at 4°C for 20 mins at 2000-3000 rpm to pellet debris and remove clear supernatant to clean microcentrifuge tube for ELISA or storage.

**Tissue samples:** The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1X PBS to remove excess blood & homogenize in 20ml of 1X PBS and store overnight at ≤ -20°C. Two freeze-thaw cycles are required to break the cell membranes. Centrifuge homogenates for 5 mins at 5,000xg. Remove the supernatant and assay immeduately or aliquot and store at -20°C or -80°C.

**Notes**

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (1 month) or -80°C (2 months) to avoid loss of bioactivity and contamination.

2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impact of chemical components.

3. Cell viability, cell number and sample lysis time may interfere with the detection of analytes from cell culture supernatants.

4. Sample hemolysis will influence the result, therefore hemolytic specimens cannot be detected.

5. Samples that contain NaN₃ cannot be detected as it interferes with HRP.
Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate X3 times with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute.

Sample Dilution Guidelines - Example

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

**High target protein concentration**: Dilution 1:100 (i.e. Add 1µl of sample into 99µl of Sample / Standard dilution buffer)

**Medium target protein concentration**: Dilution 1:10 (i.e. Add 10µl of sample into 90µl of Sample / Standard dilution buffer).

**Low target protein concentration**: Dilution 1:2 (i.e. Add 50µl of sample into 50µl of Sample / Standard dilution buffer)

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

1. Wash Buffer:

Dilute 30ml of Concentrated Wash Buffer into 750 ml of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. If crystals have formed in the concentrate, warm with 40°C water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.
2. Standard Dilution - Example:
Dilute each standard vial provided with 1ml standard dilution buffer to create the Standard Stock Solution. Keep tube at room temperature for 10 min and mix thoroughly.

To create the standard series, label 6 microcentrifuge tubes and aliquot 300µl of the Sample/Standard dilution buffer into each tube. Add 300µl of the standard stock solution into 1st tube and mix thoroughly. Transfer 300µl from 1st tube to 2nd tube and mix thoroughly. Repeat this dilution process until the standard series is complete (see Figure 1 below for details).

![Figure 1: Serial dilution of stock standard with 300µl to create standard for analysis.](image)

**Note:** The standard solutions are best used within 2 hours. The standard solution should be kept at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of Biotin-Detection antibody working solution:
Prepare within 1 hour before the experiment.

1. Calculate the total volume of the working solution: 50μl / well × quantity of wells (Allow 100-200μl more than the total volume).

2. Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1µl of Biotin-detection antibody into 99µl of Antibody dilution buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:
Prepare within 30 minutes of starting the experiment.

1. Calculate the total volume of the working solution: 100µl / well × quantity of wells. (Allow 100-200µl more than the total volume)

2. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1µl of SABC into 99µl of SABC dilution buffer.)
7. ASSAY PROCEDURE

Equilibrate the SABC working solution and TMB substrate for at least 30 min at 37°C.
When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (zero) wells.

2. Add Sample and Biotin-detection antibody: Add 50µL of Standard, Blank (Sample/Standard dilution buffer), or Sample per well. The blank well is added with Sample / Standard dilution buffer. Immediately add 50 µL of Biotin-detection antibody working solution to each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37°C. (Solutions are added to the bottom of micro ELISA plate well, avoid touching plate walls and foaming).

3. Wash: Aspirate each well and wash, repeating the process x3 according to instructions.

4. HRP-Streptavidin Conjugate (SABC): Add 100µL of SABC working solution to each well. Cover with a new Plate sealer. Incubate for 30 minutes at 37°C.

5. Wash: Repeat the aspiration/wash process for x5 times according to instructions.

6. TMB Substrate: Add 90µL of TMB Substrate to each well. Cover with a new plate sealer. Incubate for about 10-20 minutes at 37°C. Protect from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. When apparent gradient appears in standard wells, you can terminate the reaction.

7. Stop: Add 50µL of Stop Solution to each well. Wells will turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.

8. OD Measurement: Determine the optical density (OD Value) of each well at once, using a micro-plate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters.
8. CALCULATION OF RESULTS

Calculate using the following equation:

\[ \text{The relative O.D.450} = \text{(the O.D.450 of each well)} - \text{(the O.D.450 of Zero well)} \]

The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be determined from the standard curve. It is recommended to use professional software such as curve expert 1.3.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.
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