ELISAGenie

Technical Manual

Sandwich ELISA Kit

SKU: FI

Research Use Only

1. DESCRIPTION AND PRINCIPLE

The ELISA Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

The ELISA Genie (enzyme-linked immunosorbent assays) assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, ELISA Genie have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands. Our assay kits use a quantitative sandwich ELISA technique and each kit comes with highly specific antibodies pre-coated onto a 96-well microtiter plate.

At ELISA Genie we understand the need for speed! Therefore, we have developed an ultrafast protocol for rapid results. Once you have prepared and plated your samples, blanks and standards, you simply incubate with the specific biotin-conjugated primary antibody and Avidin conjugated Horseradish Peroxidase (HRP). After plate washing and addition of the TMB (3,3',5,5'-Tetramethylbenzidine) solution, the appearance of a blue colour is detected due to an enzymatic reaction catalysed by HRP. Next the addition of the Stop Solution terminates the HRP reaction and the blue colour turns yellow with the signal intensity measured on a plate reader at 450nm. The amount of bound analyte is proportional to the signal generated by the reaction meaning the kit assay gives you a quantitative measurement of the analyte in your samples.

2. KIT CONTENTS

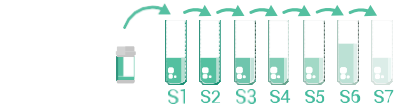
Each kit contains reagents for either 48 or 96 assays including:

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

Additional materials required:

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

3. WORKFLOW OVERVIEW



Prepare plate standards and reagents, Sample (diluted at least ½ with Sample Dilution Buffer)

Wash plate x2

Add 100µl of samples, standards and controls

90 mins

37°C

Wash plate x2

Add 100µl Biotin-labelled antibody working solution

60 mins

37°C

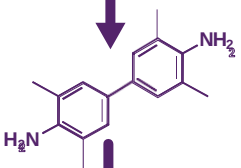
Wash plate x3

Add 100µl SABC working solution

30 mins

37°C

Wash plate x5

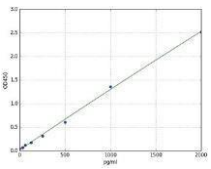


Add 90µl TMB Substrate

10-20 mins

37°C

STOP +



Add 50µl STOP solution and read absorbance @ 450nm

4. SHIPPING AND STORAGE

ELISA Genie ELISA kits are shipped in ice packs. Store kits at 4°C for up to 6 months.

LOOKING FOR TECHNICAL SUPPORT?

Email **hello@elisagenie.com** with any queries.

5. SAMPLE PREPARATION

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 twice 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 lyses 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 Homogenates: As hemolysis blood can have an impact on assay result, it is necessary to remove residual blood by washing the tissue with pre-cooling PBS buffer (0.01M, pH=7.4).Mince tissue after weighing it and homogenize in PBS (the volume depends on the weight of the tissue. Usually, 9mL PBS would be appropriate for 1 gram tissue pieces. Some protease inhibitors are recommended to add to the PBS) with a glass homogenizer on ice. To further breakdown the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g in order to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Notes

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

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

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

4. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

6. STANDARD & REAGENT PREPARATION

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

Note: Refer to datasheet provided with the kit for comprehensive sample dilution guidelines.

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

Bring all reagents to room temperature before use.

1. Wash Buffer:

Dilute 30ml (15 ml for 48T) of Concentrated Wash Buffer into 750 ml (375 ml for 48T) 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:

Note: Refer to datasheet provided with the kit for comprehensive sample dilution guidelines.

Dilute each standard vial provided with 1ml sample standard dilution buffer to create the Standard Stock Solution. Keep tube at room temperature for 10 min and mix thoroughly.

6. STANDARD & REAGENT PREPARATION

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).

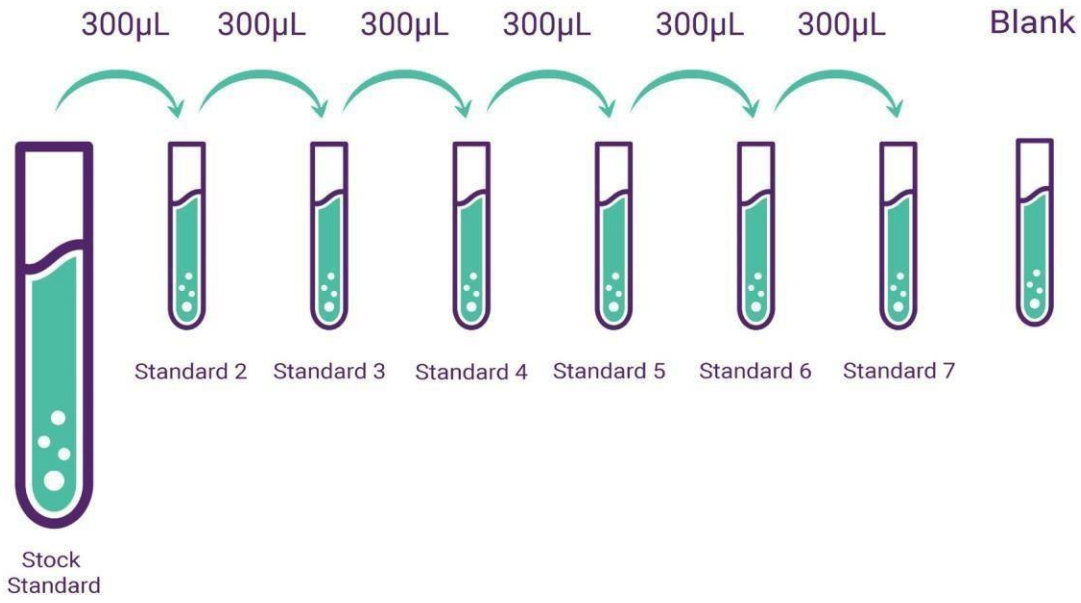


Figure 1: Serial dilution of stock standard with 300µl to create standard dilution series for analysis.

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: 100µl / 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

Before adding to the wells, equilibrate the SABC working solution and TMB substrate for at least 30 mins at room temperature. When diluting samples and reagents, ensure they are mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standard, test sample (diluted at least ½ with Sample Dilution Buffer) and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. Wash plate twice before adding standard, sample and control (zero) wells! For correct washing procedure, see Page 7, Manual Washing & Automated Washing.

2. Aliquot 100µl of standard solutions into the standard wells.

3. Add 100µl of Sample / Standard dilution buffer into the control (zero) well.

4. Add 100µl of properly diluted sample (serum, plasma, tissue homogenates and other biological fluids.) into test sample wells.

5. Seal the plate with a cover and incubate at 37 °C for 90 mins.

6. Remove the cover, aspirate the liquid from the plate and wash plate twice with Wash Buffer according to instructions detailed on Page 7. Do not let the wells dry out at anytime.

7. Add 100µl of Biotin-detection antibody working solution to the bottom of each well (standard, test sample & zero wells) without touching the side walls.

8. Seal the plate with a cover and incubate at 37°C for 60 mins.

9. Remove the cover, and wash plate 3 times with Wash Buffer.

10. Add 100µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 mins.

11. Remove the cover and wash plate 5 times with Wash Buffer.

12. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark for 10-20 mins. (Note: This incubation time is for reference only, the optimal time should be determined by the end-user.) As soon as a blue color develops in the first 3-4 wells (with most concentrated standards) and the other wells show no obvious color, terminate the reaction by moving to Step 13.

13.Add 50µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

14. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the Stop solution.

8. CALCULATION OF RESULTS

Calculate using the following equation:

The relative O.D.450 = (the O.D.450 of each well) – (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.

NOTES

Where to find Us?

Email: hello@elisagenie.com

Phone: + (44) 2081237624

Address: 2 Duke
Street
London
United Kingdom
W1U 3EH

Unit G1
The Steelworks
Foley Street
Dublin 1
Ireland