Demonstration of hormone assay through ELISA from available teaching kit

Prepared by Dr. Sagar Adhurya, Assistant Professor in Zoology, West Bengal Education Service (WBES)

Objective

- To demonstrate the quantification of a specific hormone using the ELISA (Enzyme-Linked Immunosorbent Assay) methodology.
- To familiarise students with assay principles, including antigen–antibody binding, signal generation via enzyme–substrate reactions, and data interpretation using a standard curve.

Introduction

ELISA (Enzyme-Linked Immunosorbent Assay) is a sensitive immunological technique used to detect and quantify specific biomolecules such as **hormones**, **proteins**, or **antibodies** in a sample. In this demonstration, a teaching kit is used to simulate the detection and quantification of a hormone. The assay is designed to provide hands-on experience with sample handling, incubation protocols, washing steps, and the interpretation of colorimetric changes that are proportional to the hormone concentration. While various hormone targets (e.g., insulin, cortisol, thyroxine) are possible, the kit typically focuses on one for instructional clarity.

Principle

Today
Create practical write up for this: Demonstration of hormone assay through ELISA from available teaching kit
Below is a detailed, step-by-step practical write-up for demonstrating a hormone assay using an ELISA teaching kit. This demonstration is intended to highlight the analytical power of ELISA in quantifying hormone levels in biological samples and to show the fundamental principles of immunoassays in a teaching laboratory setting.

1. Title

Demonstration of Hormone Assay by ELISA Using a Teaching Kit

2. Objective

- To demonstrate the quantification of a specific hormone using the ELISA (Enzyme-Linked Immunosorbent Assay) methodology.

- To familiarize students with assay principles including antigen–antibody binding, signal generation via enzyme–substrate reactions, and data interpretation using a standard curve.

3. Introduction

ELISA is a sensitive immunoassay technique widely used in both research and clinical diagnostics. In this demonstration, a teaching kit is used to simulate the detection and quantification of a hormone. The assay is designed to provide hands-on experience with sample handling, incubation protocols, washing steps, and the interpretation of colorimetric changes that are proportional to the hormone concentration. While various hormone targets (e.g., insulin, cortisol, thyroxine) are possible, the kit typically focuses on one for instructional clarity.

4. Principle of the Assay

The assay is based on these key immunological concepts:

- **Antigen-Antibody Interaction:** The hormone (antigen) in the sample specifically binds to pre-coated capture antibodies on a microplate.
- **Detection System:** After the initial binding, a secondary antibody conjugated with an enzyme (commonly horseradish peroxidase or alkaline phosphatase) is introduced, which binds to the hormone forming a sandwich complex.
- **Signal Generation:** Upon addition of a chromogenic substrate (e.g., TMB for horseradish peroxidase), the enzyme catalyzes a reaction leading to a color change. The intensity of the color is directly proportional to the hormone concentration in the sample.
- **Quantification:** The colorimetric output is measured with a spectrophotometer (typically at 450 nm), and the hormone concentration is then determined by comparing the results to a standard curve generated using known hormone concentrations.

Materials and Equipment

Reagents and Kits

- **ELISA Plate:** Pre-coated with capture antibodies specific to the hormone.
- **Hormone Standards:** A series of known concentrations used to generate a calibration curve.
- **Sample Diluent:** For diluting samples to the optimal concentration.
- **Wash Buffer:** To remove unbound reagents between steps.
- **Detection Antibody:** Enzyme-labelled, specific for the hormone.
- **Substrate Solution:** Typically, TMB (3,3’,5,5’-Tetramethylbenzidine) for colour development.
- **Stop Solution:** Often an acid (e.g., sulfuric acid) to halt the enzymatic reaction.

Laboratory Equipment

- Micropipettes and sterile tips

- Microplate washer or manual pipetting tools for washing
- A microplate reader (spectrophotometer) for absorbance measurement at the appropriate wavelength (usually 450 nm)
- Incubator (if temperature control is required during incubation steps)
- Personal Protective Equipment (PPE): lab coat, gloves, safety goggles

Experimental Procedure

A. Preparation

1. **Reagent Equilibration:** Allow all reagents, including the ELISA kit components and samples, to come to room temperature.
2. **Reconstitution of Reagents:** Reconstitute any lyophilized standards or detection antibodies according to the kit's instructions.

B. Plate Setup

1. **Standards and Samples:**
    - Label wells for standards and unknown samples.
    - In duplicate or triplicate, add 50–100 µL of each standard and sample into appropriate wells to ensure reliable data.
2. **Incubation:**
    - Cover the plate and incubate for the recommended time (generally 1–2 hours) to allow the hormone to bind to the capture antibodies.

C. Washing

1. **First Wash Cycle:**
    - After incubation, aspirate the liquid from the wells.
    - Add 200–300 µL of wash buffer per well.
    - Repeat the wash step 3–5 times to remove unbound material.

D. Addition of Detection Antibody

1. **Enzyme–Antibody Conjugate:**
    - Add the detection antibody solution to each well according to the kit's protocol.
    - Incubate again for 30 minutes to 1 hour as specified.
2. **Wash:**
    - Perform another round of wash cycles (typically 3–5 washes) to remove any free enzyme-conjugate.

E. Substrate Reaction

1. **Substrate Addition:**
    - Add an appropriate volume of TMB or other substrate to each well.
    - Incubate in the dark (typically 15–30 minutes) to allow colour development. The reaction time may need optimization.
2. **Stopping the Reaction:**
    - Add a stop solution (commonly 50 µL of sulfuric acid) to each well, which changes the colour from blue to yellow in a TMB assay.

F. Absorbance Measurement and Data Collection

1. **Measurement:**
    - Immediately measure the absorbance using the microplate reader at 450 nm.
2. **Standard Curve Construction:**
    - Plot absorbance versus the known concentrations of hormone from the standards.
    - Use a linear or non-linear regression model as dictated by the standard curve for accurate interpolation.
3. **Sample Concentration Determination:**
    - Calculate the hormone concentration in the unknown samples using the standard curve.

Observation table

Sample Type	OD (450 nm)	Estimated Hormone Concentration (ng/mL)
Blank (Standard 0)	0.05	0.0
Standard 1	0.25	2.5
Standard 2	0.48	5.0
Standard 3	0.92	10.0
Standard 4	1.56	20.0
Test Sample A	0.60	~7.1
Test Sample B	1.35	~16.6

Data Analysis and Standard Curve Preparation

To calculate hormone concentration in test samples:

1. Use OD values from the standards to prepare a standard curve.

Known Concentration (ng/mL)	OD at 450 nm
0 (blank)	0.05
2.5	0.25
5.0	0.48
10.0	0.92
20.0	1.56

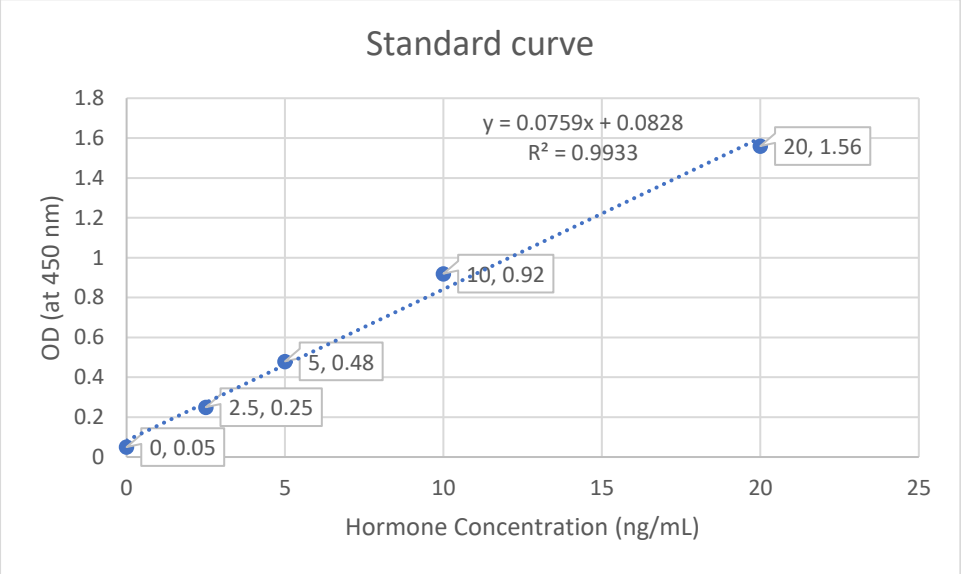
2. Plot a graph with concentration on the X-axis and OD on the Y-axis.
3. Apply linear regression to obtain the best-fit line: $OD = m \times (Concentration) + c$, where, m and c are regression coefficients.
4. Determine the coefficient of determination (R^2) to assess fit quality ($R^2 > 0.98$ is ideal).
5. Estimate unknown concentrations by plugging their OD values into the regression equation.

Result

The estimated value for the m and c is 0.0759 and 0.0828.

By using the formula $OD = m \times (Concentration) + c$, the concentrations of test samples are estimated:

$$Concentration = \frac{OD - c}{m}$$



The following result is obtained:

Test samples	**OD**	**Estimated concentration (ng/mL)**
A	0.6	6.814
B	1.35	16.696

Conclusion

The hormone assay was successfully performed using a standard sandwich ELISA method. The optical density values were used to construct a standard curve, from which hormone concentrations of unknown samples were accurately estimated using linear regression analysis.

Precautions

- Handle all biological materials and reagents with care.
- Avoid cross-contamination by changing tips between samples.
- Ensure consistent timing and temperature across wells.
- Read the plate promptly after stopping the reaction for accurate results.