Product datasheet

Note: This protocol was modified as of March 2018. All previous protocols are obsolete.

Student ELISA Kit

Product #: 0046

Storage recommendations

Store the kit at 2-8°C. The kit is stable for a period of up to 3 months from the date of receipt.

Description

This student ELISA kit enables the quantitative measurement of a model antigen in a classroom setting. The entire assay can be performed in approximately 45 minutes by the utilisation of 10 minute incubation times. Each kit contains one strip-well plate along with sufficient key reagents for plate development.

Each kit is suitable for 4 groups of students by using 3 x 8 strip-well columns per group. This will allow the standards, two positives (or unknowns; ie. 250, 50 and 0ng/ml) and a negative control to be run in duplicate. Many other formats are also possible depending on end user requirements.

Kit contents	**0046**
96 well strip-plate	1
Strip-well frames	3
Antigen standard	2 vials (5000ng/vial)
Coating buffer	28mls
20x wash Buffer	28mls
Assay diluent 1B	28mls
Anti-antigen HRP labelled detection antibody	1 vial (10µL).
TMB substrate	12mls
Stop solution	10mls

Additional materials required

- Pipettes: 10-100µL and 100-1000 µL.
    - If available a multi channel 30-300µL pipette is useful for doing the washing steps.
- 1L graduated container to prepare wash buffer.
- 1.5ml polypropylene tubes or similar for dilutions.

Reagent preparation

Wash buffer (20x)

If crystals have formed in the concentrate, warm to room temperature and mix well. Once all the visible crystals have dissolved, dilute 25ml of wash buffer concentrate in 475ml of distilled water to make 500ml of wash buffer.

Antigen

The antigen standard (5000ng) is present in lyophilised form in a 2ml microtube. Prior to reconstitution, briefly centrifuge the vial to ensure that any loose lyophilised material has collected at the bottom of the tube. Reconstitute the standard antigen in 1000µL of **coating buffer** for a final stock concentration of 5000ng/ml. **Allow the antigen to dissolve for at least 15 minutes with regular inversion prior to use.**

From this stock solution, the top antigen standard curve point (500ng/ml) and the 3 unknowns (ie. 250, 50 and 0ng/ml) can be prepared in **coating buffer** (Table 1).

Each student group can be provided with their own 1ml vial of the top antigen standard curve point (500ng/ml) and each unknown or they can acquire their own 1ml aliquot from the 5ml tubes.

Table 1. Preparation of stock solutions for 4 student groups.

	Antigen stock (5000ng/ml)	Coating buffer	Each student group receives
500ng/ml	500µL	4500µL	1x 1ml vial
Unknown 1 (250ng/ml)	250µL	4750µL	1x 1ml vial
Unknown 2 (50ng/ml)	50µL	4950µL	1x 1ml vial
Unknown 3 (0ng/ml)	0µL	5000µL	1x 1ml vial

Detection antibody

The anti-antigen-HRP labelled detection antibody is provided in a liquid form and should be diluted **2000 fold in assay diluent 1B** immediately prior to use. For example, if developing an entire plate aliquot 6µL of anti-antigen-HRP labelled detection antibody into 12ml of **assay diluent 1B**. Provide 3ml of detection antibody per student group.

Protocol

Ensure that all reagents are at room temperature prior to use.

1. Prepare solutions as required by following the instructions outlined in the reagent preparation section.

2. For 4 student groups take 9 strip wells out of the full plate and add 3 strip wells to each of the 3 strip well frames.

Generating the antigen standard curve.

3. The standard curve should be performed in duplicate starting at 500ng/ml and diluted 1 in 2 down to at least 7.81ng/ml (columns 1 and 2) in **coating buffer**. Wells H1 and H2 are the negative controls, which contains coating buffer alone.

    a. Serial dilutions are easily achieved by using 1.5ml microtubes (or similar). Set up 6 tubes and label them 250, 125, 62.5, 31.25, 15.63 and 7.81ng/ml respectively.
    b. Aliquot 250µL of coating buffer into each tube.
    c. Transfer 250 µL of the 500ng/ml antigen standard into the first tube (now 250ng/ml), mix well by pipetting up and down 4 times before transferring 250µL to the next tube (now 125ng/ml).
    d. Repeat until the serial dilution is complete.

Coating the plate with the antigen standard curve and unknown samples.

4. Add 100µL of antigen standards, unknown samples and zero standard controls to the plate. The standard curve should be loaded down the plate in columns 1 and 2 starting at 500ng/ml. Thus wells A1/A2 should contain 500ng/ml of antigen, wells B1/B2 250ng/ml of antigen and so on down the plate to wells G1/G2, which will contain 7.813ng/ml antigen. Wells H1 and H2 are the negative controls and contain coating buffer alone.

    Samples are added in duplicate in row 3 where sample 1 is in A3-B3, sample 2 is in C3-D3 and sample 3 is in E3-F3.

5. Incubate for 10 minutes at room temperature.

Plate washing.

6. Flick out the contents of the wells into a sink/bucket.

7. Wash the plate 3x with wash buffer (250µL). Washing can be achieved with a multichannel pipette, plate washer or similar. To remove residual wash buffer invert the plate and tap the plate on paper towels between each wash.

Blocking the plate

8. Add 100µL of assay diluent 1B to each well.

9. Incubate for 10 minutes.

Note: If there is any theory based learning to be conducted then this is the ideal time. An extended incubation at this point will have no effect on the outcome of the assay.

Plate washing.

10. Repeat the wash steps in step 6/7.

Adding the detection antibody.

11. Add 100µL of the anti-antigen-HRP labelled detection antibody each well. Incubate for 10 minutes at room temperature.

Plate washing.

12. Repeat the wash steps in step 6/7.

Adding the substrate

13. Add 100µL of TMB substrate to each well and incubate for 15 minutes at room temperature. Wells containing antigen will turn blue.

Developing the plate

14. Stop the reaction with 50µL of stop solution. The wells will turn from blue to yellow in colour.

15. Determine the optical density (OD) of the plate using a micro-plate reader set at 450nm. If the micro-plate reader allows wavelength correction, set at 570nm.

Calculation of results

To find the concentration of the unknown samples a standard curve must be drawn. This can be done using graph paper or a curve fitting software package.

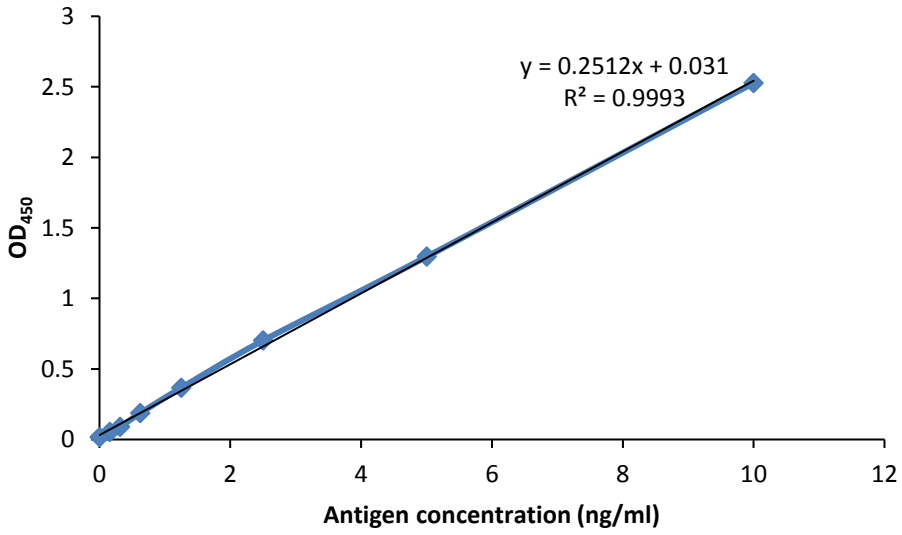
Create the standard curve by plotting the mean OD of each standard on the Y-axis versus the standard concentration on the X-axis. To find the concentrations of the unknown samples simply interpolate the OD values (Y-axis) with the concentration of antigen (X-axis).

If using a software package, it is preferable to use a program capable of generating a four parameter logistic (4-PL) curve fit. A free user friendly 4-PL program is available online at http://elisaanalysis.com. Alternatively, plot a best fit curve through the standard points and overlay a linear trend-line. The resultant equation displayed on the graph can then be used to calculate the unknown concentrations by solving for x. When fitting a trend-line it is essential that the r^2 value is 0.99 or higher. If not, then simply find the linear portion of the graph by removing the higher standard points from the analysis.

For the example below, if Y = 0.2512x + 0.031 then X = (Y-0.031)/0.2512. Thus if Y (OD_{450}) =1 then X = 3.86ng/ml.

Standard curve

This is a representative example of a typical standard curve. A separate standard curve must be generated for each experiment.



Protocol summary

Laboratory staff

Prepare reagents as per the reagent preparation section

Assemble 3 strip wells into each strip well frame

Make the top antigen standard curve point (500ng/ml) and unknown samples in coating buffer (ie. 250, 50 and 0ng/ml)

Protocol summary

Students

Make the antigen standard curve in **coating buffer**

Add 100µL of standards (duplicate) and samples (duplicate) onto the plate

Incubate for 10 minutes

Wash 3x

Block the plate by adding 100µL of assay diluent 1B to each well

Incubate for 10 minutes

Wash 3x

Add 100µL of the anti-antigen-HRP labelled detection antibody to each well

Incubate for 10 minutes

Wash 3x

Develop the plate by adding 100µL of TMB solution to each well

Incubate for 15 minutes

Stop the reaction with 50 µL of stop solution

Determine the optical density using a plate reader

Analyse results

Assay templates

	1	2	3
A			
B			
C			
D			
E			
F			
G			
H			

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												