ELISA KIT FOR DETECTING IMMUNE RESPONSES TO INCLUSION BODY HEPATITIS

5 x 96-well Test Kit

Store at 2-8°C

CONTENTS

5 x 96 well FAdv-8 antigen-coated microwell plates
1 x 100mL bottle of ELISA Diluent Concentrate 10x (blue solution)
7 x 0.08mL vials of Standards (plain caps)
1 x 0.3mL vial of anti-chicken HRP Conjugate (blue cap)
1 x 250mL bottle of Wash Buffer concentrate (20x)
1 x 3.0mL Substrate Concentrate (20x)
1 x 60mL Substrate Buffer
1 x 30mL Stopping Solution

METHOD

Preparation All steps are carried out at room temperature.

Ensure that microtitre plate and all reagents are at room temperature before use.

Quantities indicated below refer to those required for the use of ONE plate.

To prepare ELISA diluent, add 15 ml of the concentrate to 135 ml of distilled water.

To prepare washing buffer, dilute 25mL or dispense one measure from the x20 dispensing bottle into the 500 ml wash bottle and fill to the mark with distilled water.

Plate layout There is sufficient room on the plate for 80 test samples (one sample per well) and two rows for the seven standard serum samples to be added in duplicate. Please refer to the suggested Standard Plate Layout.

1. Dilution of samples Dilute test samples and each Standard Sera 1:100 by adding 10uL of sample in 1 mL of ELISA Diluent (1x). Mix well.

Pre-diluted standards and samples can be stored at 4°C up to two weeks.

2. Sample Addition. Add 50uL of each diluted sample into a well. Add 50uL of each diluted Standard Sera. Include a blank well by adding 50uL of ELISA Diluent (1x). A suggested Plate Layout is shown in Figure 1.

Standard No. 1 is a non-reactor (negative control) and Standard No. 7 is a high titre sample (positive control).

3. Incubation of plates Incubate at room temperature in a humid container for 1 hour.

4. Washing plates Wash the plates three times using 1x wash buffer. If not using an automatic plate washer, flick the plate to empty the contents and immediately use a wash bottle to wash the wells three times. Tap dry on laboratory tissue to remove residue.

5. Dilution and addition of conjugate.
Dilute the conjugate 1:120 by adding 50uL of conjugate to 6mL of ELISA Diluent (1x). Mix well.

Add 50uL of diluted conjugate to each well.

6. Incubate as in Step 3.

7. Wash plates as in Step 4.

8. Addition of TMB substrate.

Dilute the Substrate Concentrate by adding 0.6 mL of the concentrate to 11.4 mL of Substrate Buffer (enough for 1 plate). Mix well.

Add 100uL of substrate to each well.

9. Colour Development.

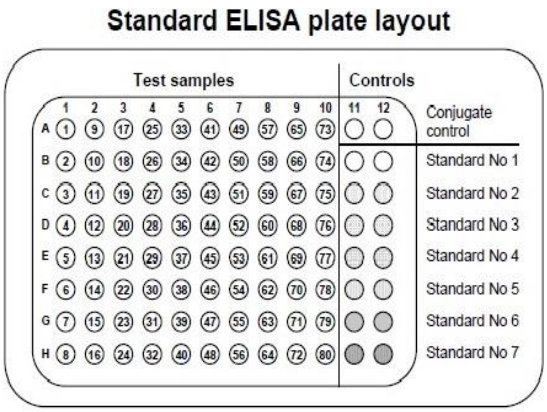
Incubate for 15 minutes in the dark at room temperature.

10. Stopping the reaction. Add 50uL of stopping solution to each well.

11. Reading

Read the strip either at the single wavelength of 450 nm or dual wavelength 450/620nm.

Figure 1. ELISA Plate Layout



INTERPRETATION OF RESULTS

A standard curve may be drawn using the optical densities of the seven samples as the Y values.

The X values can be the numbers 1 to 7 corresponding to the controls. The test samples can be allocated to groups on the basis of the optical density compared with the optical density of the standard curve samples.

Test serum value	Group
< Standard No 1	1
≥No 1 & <No 2	2
≥No 2 & <No 3	3
≥No 3 & <No 4	4
≥No 4 & <No 5	5
≥No 5 & <No 6	6
≥No 6 & <No 7	7
≥No 7	8

Alternatively the titre of the samples may be calculated by allocating the following values to the seven standard curve samples and comparing the optical densities of the test samples with the control samples.

Standard No	Titre
1	<2
2	4
3	8
4	16
5	32
6	128
7	512

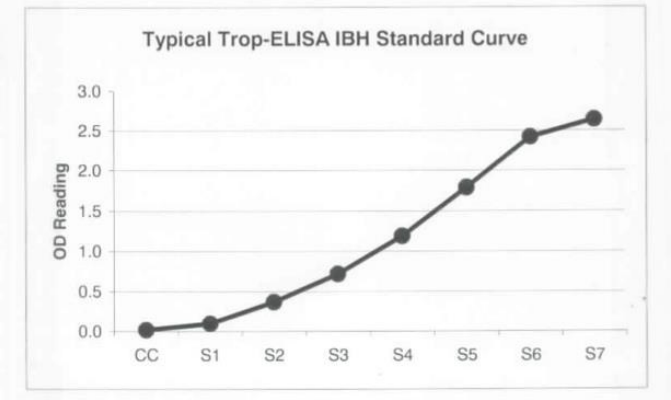


Figure 2. Expected standard curve using Standards 1-7.

Under optimum conditions, the OD reading of the Reference Controls should be:

Positive Reference Antigen (Std No. 7) = OD > 1.1
Negative Control (Standard No. 1) = OD < 0.3

Notes:

The configuration of the Trop-ELISA IBH is based on the capture of IBH viral antigen using monoclonal antibody. This has resulted in both a more sensitive and more specific assay for the detection of antibodies to IBH than a simple indirect ELISA (Ahmad, 1996)

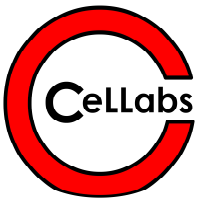
Store the kit at 2-8°C.

The standards and conjugate may be stored at -20°C if desired.

All reagents have been tested to have a storage life of at least 6 months at 2-8°C.

This kit is to be used for in vitro testing purposes only. All components must be disposed of by autoclaving at the completion of testing or dispose of in accordance with local operating regulations.

For technical support and re-order information, refer to the back of this kit insert.



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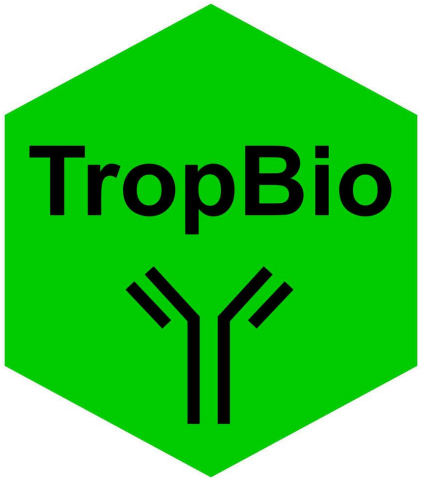
Trop-ELISA IBH



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INCLUSION BODY HEPATITIS VIRUS

(Fowl Adenovirus Serotype 8 – hypervirulent)

480 Test Kit