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## **Chicken Inclusion Body Hepatitis (IBH) ELISA Kit**

**96 Tests**

**Catalogue Number:SL0098Ch**

**Store all reagents at 2-8 °C**

**Validity Perid: six months**

**For samples:**

**In serum, plasma, culture media or any biological fluid.**

**FOR RESEARCH USE ONLY !**

**NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS !**

**PLEASE READTHROUGH ENTIRE PROCEDURE BEFORE BEGINNING**

# **Chicken Inclusion Body Hepatitis (IBH)ELISA Kit**

***FOR RESEARCH USE ONLY***

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## **Drug Names**

Generic Name: **Chicken Inclusion Body Hepatitis (IBH)ELISA Kit**

## **Purpose**

This kit allows for the determination of IBH concentrations in Chicken serum, blood plasma, and other biological fluids.

## **Principle**

The kit assay Chicken IBH level in the sample, use Purified Chicken IBH antibody to coat microtiter plate wells, make solid-phase antibody, then add IBH to wells, Combined With IBH, after washing and removing non-combinative antigen and other components ,then Combined IBH antibody which with HRP labeled become antibody - antigen - enzyme-antibody complex, after washing Completely, Add TMB substrate solution,, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. Compared with the CUTOFF value, according to this to judge IBH exist in the sample or not.

## Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	
2	Closure plate membrane	2	
3	Sealed bags	1	
4	Microelisa stripplate	1	2-8°C
5	Negative control	0.5ml×1 bottle	2-8°C
6	Positive control	0.5ml×1 bottle	2-8°C
7	HRP-Conjugate reagent	6ml×1 bottle	2-8°C
8	Sample diluent	6ml×1 bottle	2-8°C
9	Chromogen Solution A	6ml×1 bottle	2-8°C
10	Chromogen Solution B	6ml×1 bottle	2-8°C
11	Stop Solution	6ml×1 bottle	2-8°C
12	wash solution	(20ml×30 fold) ×1bottle	2-8°C

## Specimen requirements

1. serum- coagulation at room temperature 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
2. plasma-use suited EDTA or citrate plasma as an anticoagulant,mix 10-20 mins ,centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
3. Urine-collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid Reference to it.
4. cell culture supernatant-detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant,detect the composition of cells, Dilut cell suspension with PBS (PH7.2-7.4) , Cell concentration reached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
5. Tissue samples- After cutting samples, check the weight,add PBS (PH7.2-7.4) , Rapidly frozen with liquid nitrogen, maintain samples at 2-8°C after melting,add PBS (PH7.4) , Homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 r.p.m.

remove supernatant.

6. extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in  $-20^{\circ}\text{C}$  to preserve, Avoid repeated freeze-thaw cycles.
7. Can't detect the sample which contain  $\text{NaN}_3$ , because  $\text{NaN}_3$  inhibits HRP active.

## **Assay procedure**

1. Number: to sample correspond microtitration well and Number Sequence, each plate should be set feminine comparison 2 wells, masculine comparison 2 wells, blank comparison 1 well (don't add sample and HRP-Conjugate reagent to blank comparison well, other each step the operation are same).
2. add sample: separately add Positive control and Negative control  $50\mu\text{l}$  to the Positive and Negative well . add Sample dilution  $40\mu\text{l}$  to testing sample well, then add testing sample  $10\mu\text{l}$ . add sample to the bottom of ELISA plates coated well , don't touch the well wall as far as possible, and Gently mix.
3. Incubate: After closing plate with Closure plate membrane ,incubate for 30 min at  $37^{\circ}\text{C}$ .
4. Configurate liquid: 30-fold wash solution diluted 30-fold with distilled water until 600ml, and reserve.
5. washing: Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.
6. add enzyme: Add HRP-Conjugate reagent  $50\mu\text{l}$  to each well, except the blank well.
7. incubate: Operation with 3.
8. washing: Operation with 5.
9. color: Add Chromogen Solution A  $50\mu\text{l}$  and Chromogen Solution B to each well, evade the light preservation for 15 min at  $37^{\circ}\text{C}$ .
10. Stop the reaction: Add Stop Solution  $50\mu\text{l}$  to each well, Stop the reaction (the blue color change to yellow color).
11. assay: take blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 15min.

## Determine the result

Test validity: the average of Positive control well  $\geq 1.00$ ; the average of Negative control well  $\leq 0.10$ .

Calculate Critical(CUT OFF) : Critical= the average of Negative control well + 0.15.

Negative control: sample OD < Calculate Critical(CUT OFF) is Chicken IBH Negative control.

Positive control: ample OD  $\geq$  Calculate Critical(CUT OFF) is Chicken IBH Positive control.

## Important notes

1. Please according to use instruction strictly, Do not mix reagents with those from other lots.
2. The kit takes out from the refrigeration environment should be balanced 15-30 minutes in the room temperature then use, ELISA plates coated if has not use up after opened, the plate should be stored in Sealed bag.
3. washing buffer will Crystallization separation, it can be heated the water helps dissolve when dilute . Washing does not affect the result.
4. Closure plate membrane only limits the disposable use, in order to avoid the overlapping pollution
5. The substrate please evade the light preservation.
6. The test result determination must take the microtiter plate reader as a standard, when use dual-wavelength to assay, Reference wavelength is 630nm.
7. All samples, washing buffer and each kind of reject should according to infective material process. Stopp Solution is 2M sulphuric acid. You must pay attention to safe when use .

## Storage and validity

1. Storage: 2-8°C.
2. validity: Six months.