

# CinnaGen Inc.

## The Immunoenzyme for the detection of HBsAg

### Composition

Label	Reagents	Presentation
1	<b>Antibody Coated wells</b> . one plate of 96 wells coated with rabbit polyclonal antibody to HBsAg	1 Microplate
2	<b>Conjugate Diluent</b> . Green-brown buffer containing detergents and bovine proteins	1 bottle
3	<b>Conjugate Concentrate</b> . Brown fluid containing two monoclonal antibodies conjugated with horseradish Peroxidase	1 Microtube
4	<b>High Positive Control</b> . Buffer with addition of 50-100 ng/ml of HBsAg	1 bottle
5	<b>Low Positive Control</b> . Buffer with addition of 2+/- 0.7ng/ml of HbsAg	1 bottle
6	<b>Negative Control</b> .	1 bottle
7	<b>Washing solution</b> . concentrated 10 times phosphate-buffered saline with tween 20	1 bottle
8	<b>TMB Diluent</b> . Tri-sodium citrate Solution with hydrogen peroxide	1 bottle
9	<b>TMB Concentrate</b> . concentrated two times 3,3',5,5'-tetramethylbenzidine Solution	1 bottle
10	<b>Stop Solution</b> . Sulfuric acid Solution 2N	1 bottle

### **Biological properties**

The test-system intended for detection of HBsAg in human blood serum and plasma by binding to specific polyclonal antibodies that are sorbed on plate well surface and forming antigen-antibody complex which can be detected with help of Peroxidase labeled mouse monoclonal antibody conjugate in coloration test with chromogen. Declared sensitivity of the enzyme-immunoassay(EIA) test Kit Is not less than 0.2ng of HBsAg per milliliter of sample. Positive Controls are potentially infectious material, so the manipulation should be carried out with all epidemic precautions.

### **Preparation of the Reagents**

1- Washing Solution of working strength : Check this concentrate Solution for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37C until crystals dissolve, mix well. dilute this Concentrate 1:10 with distilled water. Prepare at least 250ml of washing solution for 32 wells .storage at +4C up to one month.

2- Conjugate of working strength : transfer completely Conjugate Concentrate in Conjugate Diluent, mix. Alternatively, pour the necessary volume of Conjugate Concentrate into appropriate volume of Conjugate Diluent, in count of 0.1ml per 2ml for 32 wells.this solution should be prepared before use and not to be stored.

3- Substrate Solution : Prepare the mixture of equal volumes of a TMB Concentrate and TMB Diluent by adding the colorless Diluent to a pink TMB Concentrate .Keep away from direct Sunlight. Prepare before use and use it at the same day. If the mixture has turned blue, it should be discarded and to be prepared a new one.

## Test Procedure

1. Warm up Antibody Coated wells intended for the analysis at room temperature for 15 minutes. Take Strips in necessary quantity.
2. Wash the wells twice with Washing Solution of working strength before starting the work.
3. Add 50ul of Conjugate of working strength to each well.
4. Add 100ul of analyzed samples to the wells.reserve six wells for Controls; Pipette 100ul of the Negative control into three wells, Low Positive control into two wells and High Positive control into one well.  
With entering of samples the color of solution in wells turns to blue.
5. Shake the plate by gentle tapping the side. Close the plate by a cover, place it in the humid chamber and incubate for 2 hours at 37C.
6. At the end of incubation time, wash the plate 8 times as described under wash procedure.After washing is completed , tap out any residual fluid onto absorbent paper.
7. Immediately after washing , add 100ul of Substrate solution to each well.
8. cover the plate with the clean lid and incubate for 30 minutes at 37C under humid conditions in darkness. A blue color should develop in wells containing HBsAg.
9. Stop the reaction by adding 50ul of stop Solution in each well. In "Positive" wells , the blue color will turn yellow.
10. Red the absorbance(OD) at 450nm using 620-690nm as the reference Wavelength(Two-Wave Mode).

## Calculation and interpretation

the results are to be assessed and analyzed if the mean OD of the replicates of the Negative control(MNC) does not exceed 0.2, High Positive control is more than 1.0 above OD<sub>MNC</sub> and the mean of Low Positive control exceed cut-off Value.

To assess the results of the reaction , one should calculate Cut-off Value. It is determined on the basis of MNC accordingly with one of two criteria:

a)  $OD_{MNC} + 0.04$  ; b)  $OD_{MNC} * 1.5$  The greatest one is Cut-off Value.

samples giving an OD less than Cut-off Value are considered Negative.

Samples giving an OD equal or greater than Cut-off Value are considered initially Positive. Such specimens should be retested using the original sample source.

Samples that are reactive at retesting(at least in one well) should be confirmed using confirmatory kit.

## Wash Procedure

The correct washing procedure is crucial for the successful of the test. wrong mode of washing procedure, any defects of washer and other deviations at this stage are the frequent reason of enormous results.

Before the beginning of work it is necessary to wash the washer system by distilled water and to be sure in absence of a clogging of any tubes(for aspiration or filling). Then to replace the water by the wash fluid prepared according to the instruction. if the kept working strength wash fluid is used, it is necessary to look after absence of flakes and particles.

## Rules for Automated Washer

Perform 8 cycles of washing with working strength Wash Fluid ensuring that:

1. Washing is carried out in a "plate"(not "strip") mode.
2. the "fill" volume is 340ul per well.
3. The mode of washing includes soaking stage, so that the duration of one cycle is 30-40 seconds.
4. where possible a "double aspiration" is used for the maximal removal of a liquid.

## Rules for manual Washer

1. Aspirate the first row of wells.
2. Fill this row with working strength wash Fluid.
3. Repeat this procedure for each row of wells in turn.
4. Ensure that each row of wells is left to soak for 30 seconds at least.
5. Repeat 1-4 a further 7 times.
6. Aspirate the contents of the wells. After last wash the strips are inverted and tapped on dry and clean absorbent paper.