

## **Anti-HBcAg Antibody IgG Pair**

Catalog Number: RAS-P165

Pack Size: 100 µg

**IMPORTANT: Please carefully read this manual before performing your experiment.**

**For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures**

## **INTENDED USE**

This pair is developed for detection of HBcAg in samples. It is intended for research use only (RUO).

## **PRINCIPLE OF THE ASSAY**

This assay Pair is used to measure the levels of HBcAg by employing a standard sandwich-ELISA format. Firstly, attach the HBcAg Capture Antibody (Species: Mouse, Tag: Mouse IgG1 | Mouse Kappa) to the microplate, add your samples to the plate, incubate and wash the wells. Then add the Biotinylated HBcAg Detection Antibody (Species: Mouse, Tag: Mouse IgG1 | Mouse Kappa) to the plate, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of HBcAg bound.

## **MATERIALS PROVIDED**

**Table1. Materials provided**

Catalog	Components	Size (100 µg)	Format	Storage	
				Unopened	Opened
RAP165-C01	HBcAg Capture Antibody	100 µg	Powder	-20°C ~ -70°C	-70°C
RAP165-C02	Biotinylated HBcAg Detection Antibody	100 µg	Powder	-20°C ~ -70°C	-70°C

## **SRORAGE**

1. The unopened product is stable for 36 months from the date of manufacture if stored at -20°C to -70°C.
2. The opened product should be stored per Table 1. The shelf life is 90 days from the date of opening.

*Note: a. Do not use reagents past their expiration date.*

*b. Find the expiration date on the outside packaging.*

## **OTHER MATERIALS & SOLUTIONS REQUIRED**

- 1.96 well microplates: Corning , Catalog# 42592
- 2.Coating Buffer (1xCBS): 0.015mol/L Na<sub>2</sub>CO<sub>3</sub>, 0.035mol/L NaHCO<sub>3</sub>, 0.0077mol/L NaN<sub>3</sub>, pH9.59
- 3.1xWashing Buffer(1xPBST): 0.05% Tween-20 in TBS, pH7.4
- 4.Blocking Buffer: 2% BSA in 1xWashing Buffer
- 5.Dilution Buffer: 0.5% BSA in 1xWashing Buffer
- 6.Substrate Solution: InnoReagents、 Catalog # TMB-S-004
- 7.Stop Solution: 2N H<sub>2</sub>SO<sub>4</sub>
- 8.Streptavidin-HRP: Streptavidin Protein-HRP, Horseradish peroxidase conjugated Streptavidin (ACRO, Cat# STN-NH913)

## **REAGENT PREPARATION**

Bring all reagents and samples to room temperature (20°C-25°C) before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, and the size of the aliquot should not be less than 10 µg.

**Table 2. Preparation method**

ID	Components	Size (100 µg)	Storage solution concentration.	Reconstituted water Vol.
RAP165-C01	HBcAg Capture Antibody	100 µg	500 µg/mL	200 µL
RAP165-C02	Biotinylated HBcAg Detection Antibody	100 µg	200 µg/mL	500 µL

## **RECOMMENDED SAMPLE PREPARATION**

### **1. Coating**

Dilute HBcAg Capture Antibody stock solution (500 µg/mL) to 1.0 µg/mL with Coating Buffer to make HBcAg Capture Antibody working solution.

Add 100 µL of HBcAg Capture Antibody working solution (1.0 µg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4°C.

## 2. Washing

Remove the remaining solution by aspiration, add 300  $\mu$ L of 1 $\times$ Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1 $\times$ Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

## 3. Blocking

Add 300  $\mu$ L Blocking Buffer to each well, seal the plate with microplate sealing film and incubate at room temperature for 2.0 hours.

## 4. Washing

Repeat step 2.

## 5. Add Samples

Add 100  $\mu$ L Samples to each well. For blank Control wells, please add 100  $\mu$ L Dilution Buffer.

*Note: It is recommended to set doable holes for samples and standard curves to be tested.*

## 6. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

## 7. Washing

Repeat step 2.

## 8. Add Biotinylated HBcAg Detection Antibody

Dilute Biotinylated HBcAg Detection Antibody stock solution (200  $\mu$ g/mL) to 1.0  $\mu$ g/mL with Dilution Buffer to make Biotinylated HBcAg Detection Antibody working solution.

For all wells, add 100  $\mu$ L Biotinylated HBcAg Detection Antibody (1.0  $\mu$ g/mL) working solution. Please prepare it for one-time use only.

## 9. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

## 10. Washing

Repeat step 2.

## 11. Add Streptavidin-HRP

For all wells, add 100  $\mu$ L Streptavidin-HRP (dilute at 1:10000) working solution. Please prepare it for

one-time use only, avoid light.

## 12. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

## 13. Washing

Repeat step 2.

## 14. Substrate Reaction

Add 100  $\mu$ L Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

## 15. Termination

Add 50  $\mu$ L Stop Solution to each well, and tap the plate gently to allow thorough mixing.

*Note: The color in the wells should change from blue to yellow.*

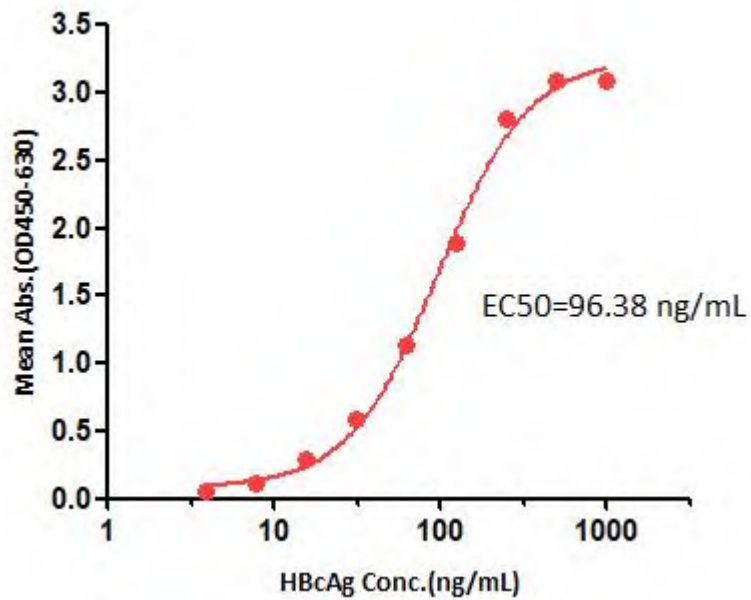
## 16. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

*Note: To reduce the background noise, subtract the value read at OD450nm with the value read at OD630 nm.*

## **TYPICAL DATA**

This data is for reference only, users need to do their own method development according to the actual situation.



Immobilized HBcAg Capture Antibody (Cat. No. RAP165-C01) at 1.0 µg/mL (100 µL/well) can bind HBcAg, and then add Biotinylated HBcAg Detection Antibody (Cat. No. RAP165-C02) at 1.0 µg/mL (100 µL/well). Detection was performed using HRP-conjugated streptavidin with a linear range of 3.9-125 ng/mL (QC tested).