ELISA Kit for Hepatitis B Surface Antigen (GoldMag-AS Magnetic Affinity Immunoassay)

Introduction

The ELISA Kit for Hepatitis B Surface Antigen (HBsAg) is an in vitro enzyme immunoassay for the detection of HBsAg in human serum or plasma. Compared with traditional ELISA method, GoldMag-AS microparticles are used as the solid phase instead of microtiter plate in this kit. Incubate GoldMag-AS microparticles coated with Anti-HBs monoclonal antibody (McAb), serum sample, and Anti-HBs polyclonal antibody (PcAb) conjugated with horseradish peroxidase (HRP) in the tubes, a sandwich complex of Anti-HBs-HBsAg-Anti-HBs labeled with HRP forms on the surface of GoldMag-AS microparticles if HBsAg is present in the sample. Then wash the GoldMag-AS microparticles and magnetically separate them from the solution to remove the unbounded serum components. Incubate with substrates (TMB) to form a colored product. Stop the reaction with 2 M H_2SO_4, and measure the absorbance at 450 nm. This kit includes 48 tests for the detection of HBsAg in human serum or plasma.

Features

- Fast – The test can be accomplished within 1 hour
- Precise – All the positive and negative samples can be detected correctly
- Simple – Easy to operate compared to traditional ELISA kit

Applications

Detection of HBsAg in human serum or plasma. For research use only.

Description

ELISA Kit for Hepatitis B Surface Antigen (HBsAg) is an in vitro enzyme immunoassay for the detection of HBsAg in human serum or plasma using GoldMag-AS microparticles as carriers. Components in this kit are prepared with pure chemicals according to the proprietary technology. One kit is consisted of eight reagents enough for 48 assays. The test is specific, sensitive, reproducible and easy to operate.

Quality Control

This kit has been used to go through the test of standard serum panel. The sensitivity of the test is 0.5 ng/ml(adr), 1.0 ng/ml(adw), 1.0 ng/ml(ay). As for the specificity of the test, the average OD of 20 normal negative samples is below 0.050. And the precision test shows that the coefficient of variability (CV) is less than 15% (n=10).

Contents

Item	Component	Amount	Part No.
1	GoldMag-AS Microparticles coated with Anti-HBs McAb	1 ml	
2	Enzyme Conjugant	3 ml	
3	Positive Control Serum	0.5 ml	
4	Negative Control Serum	0.5 ml	
5	10× Wash Buffer (dilute with distilled water to 1× prior to use)	10 ml	
6	Substrate A	3 ml	
7	Substrate B	3 ml	
8	Stop Solution	3 ml	

Storage and Stability

Store the kit at 2~8 °C. Do not freeze or dry the GoldMag-AS microparticles coated with Anti-HBs McAb. The kit is stable for six months under proper storage condition.

Additional Equipment and Materials Required

- Magnetic Separator: 48-tube magnetic separator provided by Biochain
- Pipette
- Incubator
- Microplate Reader

Protocol

The procedure described below is for the detection of HBsAg in human serum or plasma using GoldMag-AS microparticles.

1. Shake the GoldMag-AS microparticles coated with Anti-HBs
2. well to make sure they are evenly suspended in solution. Then transfer 20μl to each tube.
3. Set one blank, two positive and two negative controls for each assay. Add 50 μl serum sample, positive and negative control serum into the corresponding tubes（blank tube is omitted）, then add 50 μl of enzyme conjugant into the each tubes except the blank tube, mix thoroughly, and incubate for 25 min at 37 °C.
4. Wash Procedure: Add 500 μl of 1×wash buffer to each tube, shake vigorously, magnetically separate for 2~3 min and aspirate the supernatant. Repeat 5 times.
5. Add 50 μl of substrate A and B respectively to each tube, mix thoroughly, and incubate for 5 min at 37 °C
6. Add 100 μl of stop solution into each tube, mix thoroughly.
7. Place the tubes on the rack of magnetic separator for 1 min, transfer 100 μl of supernatant to 96-well plate.
8. Measure the absorbance using microplate reader at 450/630 nm against the blank.

Precautions

1. Shake the reagents well before use.
2. Bring ELISA kit (all reagents) and samples to room temperature before use (approximately 30 min), put the remained reagents to the sealed pouch, and return to 2~8 °C immediately.

3. The NaN_3 can't be used to preserve the reagents.
4. Do not interchange reagents between kit lots.
5. Results should be read out within 5 min.
6. Resolve the 10× wash buffer at 37 °C if crystals appear, and dilute it with distilled water to 1x prior to use.
7. Handle all reagents, samples, controls as if capable of transmitting an infectious agent. It is recommended that these reagents and samples should be handled using established good laboratory working practices.
8. Do not use the kit beyond its expiration date.

Interpretation of Results

Colorimetric Method
Cut Off Value Calculation:
COV = the average OD of Negative Controls × 2.1

Positive OD_{450} of sample ≥ COV

Negative OD_{450} of sample < COV

Invalid If the OD of Positive Control is below 0.60, the result is invalid. In any event, repeat the test.

Notes If the absorbance of Negative Control is below 0.05, calculate it as 0.05. If the absorbance of Negative Control is above 0.05, calculate it as its original value.

Trouble Shooting

1. The false negative result of the test

Try to avoid the loss of magnetic particles. For example, the magnetic particles stick to the tube wall or the magnetic separation is not performed thoroughly.

2. The result for non-specific analysis is higher than normal

Make sure to wash the GoldMag-AS microparticles sufficiently after incubation, and the results should be read out within 5 min.

This Kit is for Research Use Only