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ADVANCED ® HBeAb ELISA Test Kit

INTENDED USE
The ADVANCED HBeAb Test Kit is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of Hepatitis B e-Antibody (HBeAb) in human serum or plasma.

PRINCIPLE OF THE PROCEDURE
The Advanced HBeAb Test is an ELISA based, competition immunoassay, which employs specific anti-HBeAg antibodies and Hepatitis B e-antigen: one antibody immobilized at the bottom of the microtiterwells, and the other antibody coupled with horseradish peroxidase (HRP) as the conjugate solution. During the assay, existing HBeAb in the specimen will compete with these antibodies coupled with HRP to form an "antibody-HBeAg-antibody-HRP" or "antibody-HBeAg-antibody" immuno-complex. After the unbound material is washed off during the assay procedure, substrate is applied to indicate the test result. The appearance of blue color in microtiterwells indicates HBeAb non-reactive result. The absence of the color indicates sreactive result in the specimen.

CONTENTS OF THE KIT

		96 T/ 12wells	96T/ 8wells
1.	Coated Microtiter Well Plate	96 T/ 12wells	96T/ 8wells
2.	Enzyme Conjugate	7 ml	9 ml
3.	HBeAg reagent	7 ml	9 ml
4.	Positive Control	1 ml	1 ml
5.	Negative Control	1 ml	1 ml
6.	Color A (H_2o_2 Solution)	7ml	14ml
7.	Color B (TMB Substrate)	5 ml	14 ml
8.	Stop Solution (2M Sulfuric Acid)	7 ml	14 ml
9.	Concentrated Wash Buffer (20×)	25 ml	25 ml
10.	Plate Sealer	2 pcs	2 pcs
11.	Plastic Bag	1 pc	1 pc
12.	Package insert	1 version	1 version

(The above items should be stored at 2~8℃.)

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipettes capable of delivering 1~1000µl
2. Pipette tips
3. 37℃ incubator
4. Deionized or distilled water
5. Microtiterwell plate washer
6. Microtiterwell Plate or Strips reader with 450nm measuring wavelength and reference filter(615-690nm)

WARNINGS AND PRECAUTIONS
1. THIS PRODUCT IS FOR *IN VITRO* DIAGNOSIS ONLY.
2. Only physicians or medical technicians should handle this reagents kit.
3. Shake each reagent gently before testing.
4. Do not use kit beyond the expiration date.
5. Do not mix reagents from different lots.
6. Avoid microbial contamination of reagents.
7. *Avoid exposing TMB solution to strong light, metal, or oxidants. It should be colorless. Otherwise, it should be discarded.*

SAFETY INSTRUCTIONS

1. *WARNING-POTENTIAL BIOHAZARDS MATERIAL: THIS KIT CONTAINS HUMAN BLOOD COMPONENTS. HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS. No known test method can offer complete assurance that products derived from human source material will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious, it is recommended that these reagents and human specimens be handled with established good laboratory working practices. The Positive Control has been inactivated by heat treatment.*
2. Do not smoke or eat in areas where specimens or kits are handled.
3. Do not pipette by mouth. Wear PVC disposable gloves when handling reagents or specimens, and wash hands thoroughly with *5%* sodium hypochlorite,
4. Infectious specimens and nonacid-containing spills should be wiped up thoroughly with 5%sodium hypochlorite.
5. All waste materials should be properly disinfected before disposal. Both liquid and solid waste should be autoclaved at 121℃ for at least one hour. Solid waste can also be incinerated. Nonacidic liquid waste can be diluted to a final concentration of 1.0%. Acidic liquid waste requires neutralization before similar Treatment and should stand for 30 minutes to obtain effective disinfection.
6. Avoid contact sulfuric acid with skin or mucous membranes. If it comes in contact with skin, wash with tap water immediately.

SPECIMEN COLLECTION AND STORAGE

1. ***Collect serum or plasma specimens following regular clinical laboratory procedures. Separate the serum from the clot or plasma from the red cells as soon as possible to avoid hemolysis.***
2. Specimens containing sodium azide or particulate matter may give erroneous results.
3. Specimens should be refrigerated if not used within *3* days of collecting. Avoid freezing and thawing the specimens more than 2-3 times before using.

REAGENT STORAGE
1. The kit must be stored at 2~8℃. Use up the reagents as soon as possible after the kit is unpacked.
2. Return the reagents to cold storage immediately after use.
3. If the whole plate is not completely used, cover the unused wells with the plate sealer, put it into the plastics bag along with the desiccant and store it at 2~8℃.
4. Concentrated wash buffer should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a 37~40℃ water bath till the crystal dissolves.

PREPARATION OF REAGENTS

Wash buffer:
   a. Dilute 1 volume of concentrated wash buffer with 19 volumes of de-ionized or distilled water. Mix well
   b. Wash buffer may be stored at room temperature for 1 week.

ASSAY PROCEDURES
1. Bring all reagents and specimens to room temperature (18~25℃) before the assay. Swirl gently before use. Adjust incubator to 37±1℃.
2. Write down the numbers of specimens and the wells on the data sheet. Two wells for blanks, five additional wells for the controls and one well for each specimen. *NOTE: Cover the unused wells with plate sealer, seal in the plastics bag with desiccant, and store at 2~8℃.*
3. Add 50µl of control (3 negative controls and 2 positive controls) and 50µl of each specimen into wells respectively (Reserve 2 wells for blanks)
4. Add 50µl enzyme conjugate into each reaction well except for the blank.

NOTE: Do not touch the edge of wells to avoid false results.

5. Gently tap the plate to thoroughly mix the liquid in the wells; do not splash liquid onto the slip.
6. Add 50µl HBeAg reagent into each reaction well except for the blank.
7. Gently tap the plate to thoroughly mix the liquid in the wells; do not splash liquid onto the slips
8. Incubate the plate at 37℃ for 60 minutes in an incubator. Balance the plate in room temperature for 5 minutes.
9. Wash each well five times with wash buffer:
   a. Washing must be performed strictly according to the instructions, incomplete washing may bring out the false result.
   b. Aspirate the well contents completely into a waste flask. Then fill up the wells with wash buffer (350µl or more), avoid overflow. Allow to soak(approx. 30-60seconds). Aspirate completely and repeat the wash and soak procedure four additional times for a total of five washes.
   c. Make sure that no fluid remains on the strip-holder and strips after the last aspiration (e.g., by blotting with absorbent tissue).

   NOTE: Improper washing may cause false result.
10. Add 50µl color A and 50µl color B to each well.
11. Incubate the plate at 37℃ for 15 minutes.
12. Add 50µl 2M sulfuric acid into each well, gently tap the plate
13. Measure OD with an ELISA reader at 450nm(single wavelength) or 450nm and 630nm as reference (dual wavelength).

CALCULATIONS AND RESULT

1. Negative Control Mean absorbance (NCx):
   NCx=(NC1+NC2+NC3)/3 Eliminate any NC less than 0.5

2. Cut-off value:
   Cut-off=0.2×NCx

3. Divide the sample absorbance by the cut-off value
   Positive: sample absorbance is less than or equal to （≤） Cut-off value
   Negative: sample absorbance is greater than （>） cut-off value.

ANALYTICAL SENSITIVITY (LIMIT DETECTABILITY)

4NCU/ml

INTERPRETATION OF RESULTS

Repeat testing in duplicate of a specimen found reactive by the screening procedure will verify whether it is repeatably reactive. If neither of the repeat tests is reactive, the specimen should be considered negative. If the specimen is reactive in either of the repeat tests, the sample should be considered repeatably reactive and tested by a confirmatory test.

False reactive results may be caused by:

a. Carryover of a highly reactive sample due to contamination with of equipment or pipet tips.
b. Substrate contamination with metal ions.
c. Cross-contamination.
d. Inadequate wash or aspirations during wash procedure.
e. Failure to remove excess moisture from the bottom of the well.

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InTec PRODUCTS, INC. (XIAMEN)