Instruction Manual

HBsAg Subtype EIA

EIA based hepatitis B surface antigen subtyping kit with monoclonal antibodies

- Thoroughly read this instruction manual before use of this kit.
- This kit is for research use only.

I. Kit components

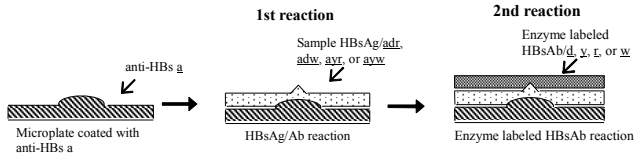
1. Microplate coated with anti-HBs a (8 wells/strip x 12) .....1 plate
2. HBsAg negative control blue..... 1 mL x 1 vial
3. HBsAg positive control (d) green..... 0.5 mL x 1 vial
4. HBsAg positive control (y) yellow..... 0.5 mL x 1 vial
5. HBsAg positive control (r) red..... 0.5 mL x 1 vial
6. HBsAg positive control (w) colorless..... 0.5 mL x 1 vial
7. Enzyme labeled monoclonal antibody (d) green .... 1.5 mL x 1 vial
8. Enzyme labeled monoclonal antibody (y) yellow... 1.5 mL x 1 vial
9. Enzyme labeled monoclonal antibody (r) red ..... 1.5 mL x 1 vial
10. Enzyme labeled monoclonal antibody (w) white.... 1.5 mL x 1 vial
11. Enzyme substrate..... 10 mL x 1 vial
    (3, 3', 5, 5'-tetramethylbenzidine)
12. Reaction stopper..... 10 mL x 1 vial
13. 20x concentrated washing solution.....25 mL x 2 vials
    (containing detergent)
14. Plate seal.....5 sheets

II. Application

Determination of subtypes of hepatitis B surface antigen (HBsAg) in serum or plasma

III. Assay principle

This kit has been developed as a reagent for research purposes to detect respective subtypic determinants, d, y, r, and w, in hepatitis B surface antigen (HBsAg) positive samples for identification of HBsAg subtypes such as adr, adw, ayr and ayw. Detection of subtypic determinants is based on the solid-phase sandwich EIA (Enzyme Immuno Assay). 96 wells of the microplate are coated with monoclonal antibody against the common determinant a of HBsAg. HBsAg in samples dispensed to wells is caught on the solid phase and their subtypic determinant, d, y, r, or w, is detected by peroxidase-labeled monoclonal antibody against corresponding determinant.



IV. Operation

1. Preparation of the washing solution.

1) Dilute 20 times 20x concentrated washing solution with purified water. Keep this solution at 2 ~ 10°C.

2. Materials required but not provided

1) Micro pipettes, 50 µL and 100 µL
2) Measuring cylinder, 500 mL
3) Aspirator (or plate washer)
4) Incubator capable of controlling temperature at 37 ± 1°C
5) Dark box (A light tight cupboard or a drawer will do.)
6) Dual wavelength microplate reader (main wavelength 450 nm and sub wavelength 620 nm or longer)

3. Assay procedure

Make sure to return the kit to 15 ~ 30°C before use.

1) Addition of samples and the control sera

Dispense 50 µL each of samples and the control sera in respective wells. Following chart shows a recommended pattern of wells assigned to samples and the control sera. Wells can be assigned in any way, however.

An example of well layout

Note:

Microplate can be divided into 12 strips. Strips not used should be kept in an aluminum pouch together with desiccant and stored at 2 ~10°C.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	B	B	6	6	6	6	14	14	14	14
B	N	N	N	N	7	7	7	7	15	15	15	15
C	P-d	P-y	P-r	P-w	8	8	8	8	16	16	16	16
D	1	1	1	1	9	9	9	9	17	17	17	17
E	2	2	2	2	10	10	10	10	18	18	18	18
F	3	3	3	3	11	11	11	11	19	19	19	19
G	4	4	4	4	12	12	12	12	20	20	20	20
H	5	5	5	5	13	13	13	13	21	21	21	21

B: Blank
N: Negative control
1-21: Sample
P-d: Positive control d
P-y: Positive control y
P-r: Positive control r
P-w: Positive control w

detection of determinant d
detection of determinant y
detection of determinant r
detection of determinant w
determinant d
determinant y
determinant r
determinant w
determinant d
determinant y
determinant r
determinant w

2) 1st reaction

Cover the microplate with the plate seal to prevent evaporation and leave the microplate to stand for 3 hrs at 37°C or 16 ~ 24 rs at 15 ~ 30°C. Incubation for 16 ~ 24 hrs at 15 ~ 30°C assures higher absorbance.

3) Washing

Remove the plate seal from the microplate.

Remove samples and microplate contents from all wells with an aspirator. Fill wells with the washing solution, turn the microplate upside down, and shake out the washing solution. epeat this 5 times.

Hold the microplate upside down and tap it against clean paper towel to thoroughly remove the washing solution from all wells.

Note:

While washing the microplate, care should be taken not to dry the microplate well surface. After washing the microplate, immediately follow the following step.

4) Addition of the labeled antibody

Dispense Enzyme labeled monoclonal antibody against determinant d, y, r or w in corresponding wells in the following manner.

Dispense 50 µL each of the labeled monoclonal antibody against;

determinant d to wells for detecting determinant d,
determinant y to wells for detecting determinant y,
determinant r to wells for detecting determinant r, and
determinant w to wells for detecting determinant w.

Note:

Do not add the labeled monoclonal antibodies in the blank wells.

5) 2nd reaction

Cover the microplate with the plate seal and incubate for 2 hrs at 37°C.

6) Washing
Repeat washing as in **3)** above.

7) Addition of Enzyme substrate
Add 100 µL of Enzyme substrate in all wells.

8) Enzyme reaction
Cover the microplate with the plate seal and incubate at 15 ~ 30°C for 30 min in the dark.

9) Addition of Reaction stopper
Remove the plate seal and stop color development by adding 100 µL of Reaction stopper in all wells.

10) Absorbance measurement
Measure absorbance of each well by a microplate reader (main wavelength 450 nm and sub wavelength 620 nm or longer).

Note:

•Adjust the zero point using the blank well.

•Absorbance must be measured within 30 min after stopping color development.

V. Interpretation of the results

1. Positive and negative determination

Calculate the Cut-off value, that is, the absorbance of the Negative Control + 0.2.

Negative: Absorbance of samples < Cut-off value
Positive: Absorbance of samples ≥ Cut-off value

2. Determination of HBsAg subtypes

1) When one of determinant d and y, or r and w (compound subtypes) are detectable, subtypic determinants should be detected as subtype corresponding to the determinant. (For example, when the case of "d:+", "y:-", "r:+", "w:-" should be determined as subtype "adr.")

2) When more than 3 of determinant d, y, r and w (compound subtypes) are detectable, majorities of determinant d or y, and r or w should be determined in general. If reactivities of them are alike, both compound subtypes should be determined.

Note:

1) When HBsAg titer of samples is high, subtypic determinants can be easily detected. When the titer is low, however, their detection sometimes becomes difficult.

2) Generally, samples test positive for either determinant d or y, as well as for either determinant r or w, corresponding to the four major HBsAg subtypes, adr, adw, ayr, or ayw. In rare samples, however, more than 3 of d, y, r and w (compound subtypes) are detectable.

VI. Warnings and precautions

This kit must be used according to the instructions and for the purpose described in this manual. No result is guaranteed in any use or for any purpose other than those described in this manual.

1. General precautions

1) Check accuracy of tools and properly use them according to their instructions.
2) Do not use a kit stored in frozen condition, because any result is not guaranteed.
3) Make sure to return the kit to 15 ~ 30°C before use.
4) Do not mix reagents of different production lots. Microplate wells can not be reused.
5) Do not use expired reagents.
6) Avoid contamination of the kit reagents with microorganisms.
7) Materials to be used for the assay must be clean and thoroughly washed with purified water in advance.
8) Replace micropipette tips for each sample and reagent.

2. Operational precautions

1) Measure blank and negative and positive controls for each assay.
2) Once assay is started, all operation must be finished promptly within specified time.
3) Absorbance must be measured within 30 min after stopping the enzyme reaction.
4) Do not scrape or touch the bottom of wells or do not dry the surface of the wells during assay.

3. Handling precautions

1) Avoid contact of reagents. If they contact skin, wash with plenty of water. Get medical care if need.
2) HBsAg positive and negative controls provided with this kit and samples should be handled as if they were potentially infectious with HBV, HCV, or HIV. Wear disposable globes and thoroughly wash hands after assaying. Do not pipette with mouth.
3) Before discarding, treat samples, reagents and materials in either of the followings.
   a) Immerse in 0.05 w/v% formalin for over 72 hrs at 37°C.
   b) Immerse in 2 w/v% glutaraldehyde solution for over 1 hr.
   c) Immerse in sodium hypochlorite solution (more than 0.1%) for over 1 hr.
   d) Autoclave for over 20 min at 121°C.
4) The HBsAg positive and negative control sera contain sodium azide and should be washed down with a sufficient volume of water to prevent formation of explosive metal azide.
5) If sample or used reagents are spilled, wipe by using sodium hypochlorite solution (Concentration of effective chlorine: 1,000 ppm or more) or glutaraldehyde (immerse in 2 w/v%, for over 1 hr), and sterilize.

VII. Storage and shelf life

Store the kit at 2 ~ 10°C and avoid freezing. This kit is stable for 1 year after the date of manufacture. Validity of kit is shown in the package.

VIII. Package

1 kit for 24 tests Code No. 1A63

IX. Reference

Usuda S, Tsuda F, et al: A solid-phase enzyme immunoassay for the common and subtypic antigen determinants of hepatitis B surface antigen with monoclonal antibodies.
J Immunol Methods **87**: 203-210, 1986.

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