HBsAg Subtype EIA
Supplement for instruction manual

For the procedure of “ad/ ay 48T ”, “ad 96T ”, and “ay 96T ”, please refer to the appendix shown below.

HBsAg Subtype EIA ad/ay 48T

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	2 vials
4.	HBsAg positive control (y) yellow	0.5 mL	x	2 vials
5.	Enzyme labeled monoclonal antibody (d) green	1.5 mL	x	2 vials
6.	Enzyme labeled monoclonal antibody (y) yellow	1.5 mL	x	2 vials
7.	Enzyme substrate (3, 3', 5, 5'-tetramethylbenzidine)	10 mL	x	1 vial
8.	Reaction stopper	10 mL	x	1 vial
9.	20x concentrated washing solution (containing detergent)	25 mL	x	2 vials
10.	Plate seal			5 sheets

IV. Operation

3. Assay procedure

1) An example of well layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	6	6	14	14	22	22	30	30	38	38
B	N	N	7	7	15	15	23	23	31	31	39	39
C	P-d	P-y	8	8	16	16	24	24	32	32	40	40
D	1	1	9	9	17	17	25	25	33	33	41	41
E	2	2	10	10	18	18	26	26	34	34	42	42
F	3	3	11	11	19	19	27	27	35	35	43	43
G	4	4	12	12	20	20	28	28	36	36	44	44
H	5	5	13	13	21	21	29	29	37	37	45	45
	detection of determinant d	detection of determinant y	determinant d	determinant y	determinant d	determinant y	determinant d	determinant y	determinant d	determinant y	determinant d	determinant y

B: Blank
N: Negative control
P-d: Positive control d
P-y: Positive control y
1…45: Samples

4) Addition of the labeled antibody

Dispense Enzyme labeled monoclonal antibody against determinant d or y 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, and
determinant y to wells for detecting determinant y.

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

V. Interpretation of the results

2. Determination of HBsAg subtypes

1) When one of determinant d and y (compound subtypes) are detectable, subtypic determinants should be detected as subtype corresponding to the determinant.

(For example, when the case of “d:+”, “y:-” should be determined as subtype “ad.”)

HBsAg Subtype EIA ad 96T

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	4 vials
4.	Enzyme labeled monoclonal antibody (d) green	1.5 mL	x	4 vials
5.	Enzyme substrate (3, 3', 5, 5'-tetramethylbenzidine)	10 mL	x	1 vial
6.	Reaction stopper	10 mL	x	1 vial
7.	20x concentrated washing solution (containing detergent)	25 mL	x	2 vials
8.	Plate seal			5 sheets

An example of well layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	6	14	22	30	38	46	54	62	70	78	86
B	N	7	15	23	31	39	47	55	63	71	79	87
C	P-d	8	16	24	32	40	48	56	64	72	80	88
D	1	9	17	25	33	41	49	57	65	73	81	89
E	2	10	18	26	34	42	50	58	66	74	82	90
F	3	11	19	27	35	43	51	59	67	75	83	91
G	4	12	20	28	36	44	52	60	68	76	84	92
H	5	13	21	29	37	45	53	61	69	77	85	93
	detection of determinant d	determinant d	determinant d	determinant d	determinant d	determinant d	determinant d	determinant d	determinant d	determinant d	determinant d	determinant d

B: Blank
N: Negative control
P-d: Positive control d
1…93: Samples

4) Addition of the labeled antibody

Dispense Enzyme labeled monoclonal antibody against determinant d 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.

Note:

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

V. Interpretation of the results

2. Determination of HBsAg subtypes

1) Subtypic determinants should be detected as subtype corresponding to the determinant.

HBsAg Subtype EIA ay 96T

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 (y) yellow	0.5 mL	x	4 vials
4.	Enzyme labeled monoclonal antibody (y) yellow	1.5 mL	x	4 vials
5.	Enzyme substrate (3, 3', 5, 5'-tetramethylbenzidine)	10 mL	x	1 vial
6.	Reaction stopper	10 mL	x	1 vial
7.	20x concentrated washing solution (containing detergent)	25 mL	x	2 vials
8.	Plate seal			5 sheets

An example of well layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	6	14	22	30	38	46	54	62	70	78	86
B	N	7	15	23	31	39	47	55	63	71	79	87
C	P-y	8	16	24	32	40	48	56	64	72	80	88
D	1	9	17	25	33	41	49	57	65	73	81	89
E	2	10	18	26	34	42	50	58	66	74	82	90
F	3	11	19	27	35	43	51	59	67	75	83	91
G	4	12	20	28	36	44	52	60	68	76	84	92
H	5	13	21	29	37	45	53	61	69	77	85	93
	detection of determinant y	determinant y	determinant y	determinant y	determinant y	determinant y	determinant y	determinant y	determinant y	determinant y	determinant y	determinant y

B: Blank
N: Negative control
P-y: Positive control y
1…93: Samples

4) Addition of the labeled antibody

Dispense Enzyme labeled monoclonal antibody against determinant y in corresponding wells in the following manner.

Dispense 50 µL each of the labeled monoclonal antibody against;

determinant y to wells for detecting determinant y.

Note:

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

V. Interpretation of the results

2. Determination of HBsAg subtypes

1) Subtypic determinants should be detected as subtype corresponding to the determinant.

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