In Vitro Diagnostic Reagent

Instruction Manual of Mycell II anti-HBs Diagnostic Reagent for Determination of anti-HBs

Thoroughly read this instruction manual before use of this kit

Background of the development and features of the kit

Hepatitis B surface antigen (HBsAg) is envelop protein of hepatitis B virus and as an infection marker, its determination is important for diagnosis of infection with hepatitis B. Determination of antibody against HBsAg is indispensable in the clinical fields, such as, in the prevention of hepatitis B infection and determination of efficacy of hepatitis B vaccine.

This kit is a reagent for determination of anti-HBs based on passive hemagglutination (PHA) using duck red blood cells as carrier coated with HBsAg. By means of control red blood cells, samples can be easily tested positive or negative in the screening test.

Features:

- Only one drop each of the sensitized and control cells is required for testing a sample allowing, by simple operation, screening of a large number of samples.
- Determination can be made in one hour.
- anti-HBs of low concentration can be determined at high sensitivity.
- Confirmatory test available with this kit assures highly reliable results.
- Quantitative determination is also available with this kit.

Kit Components

This kit consists of the following components.

1. **Red blood cells**
   Red blood cells sensitized with HBsAg
2. **Control red blood cells**
   Red blood cells sensitized with human serum
3. **Positive control**
   anti-HBs positive control (hemagglutination titer: 32 ~ 128 folds)
4. **Inhibition solution**
   Phosphate buffer solution containing purified HBsAg used for the confirmatory test of samples of which determination is suspended in the screening test
5. **Suspension solution**
   Phosphate buffer solution containing rabbit serum used for suspension of the sensitized and control cells
6. **Sample dilution buffer**
   Phosphate buffer solution containing purified horse serum used for dilution of samples

	100 tests kit	200 tests kit	1000 tests kit
Sensitized cells	0.3 mL x 1 vial	0.3 mL x 2 vials	1.4 mL x 2 vials
Control cells	0.3 mL x 1 vial	0.3 mL x 2 vials	1.4 mL x 2 vials
Positive control	0.5 mL x 1 vial	0.5 mL x 1 vial	0.5 mL x 1 vial
Inhibition solution	1 mL x 1 vial	2 mL x 1 vial	10 mL x 1 vial
Suspension solution	11 mL x 1 vial	18 mL x 1 vial	65 mL x 1 vial
Sample dilution buffer	12 mL x 1 vial	24 mL x 1 vial	55 mL x 2 vials
Dropper*	2 ea.	2 ea.	--
Vial for cell suspension	--	--	2 ea.

*Droppers for the sensitized and control cells. Not for use other than dispensing the sensitized and control cells.

Effect

Determination of serum or plasma anti-HBs.

Assay modes available

1. **Screening test**

   This test examines hemagglutination of red blood cells sensitized with HBsAg. The sensitized red blood cells hemagglutinate when anti-HBs presents in samples.

2. **Confirmatory test**

   This test is for confirmation of antigen-antibody specific hemagglutination by means of an inhibition test using the inhibition solution. In case of hemagglutination by anti-HBs in samples, when the inhibition solution is added to samples their titer is reduced due to absorption of anti-HBs by HBsAg contained in the solution. This hemagglutination inhibition, however, does not occur in non-specific hemagglutination

3. **Quantitative test**

   This test is for determination of the final hemagglutination titer of samples tested positive by the screening or confirmatory test.

Assay procedures

1. **Tools and Equipment** (not supplied with this kit)

   - V-shaped well microplate (with mesh)
   - Diluter (25µL)
   - Dropper (25µL)
   - Microplate mixer
   - Cover (for microplate)
   - Pipette (micropipette or measuring pipette)

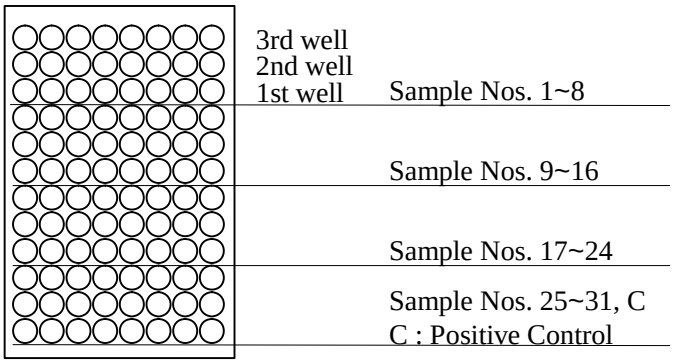
2. **Preparation of Reagents**

   After removing supernatant, add 3 mL each of the suspension solution to the sensitized and control cells and suspend.

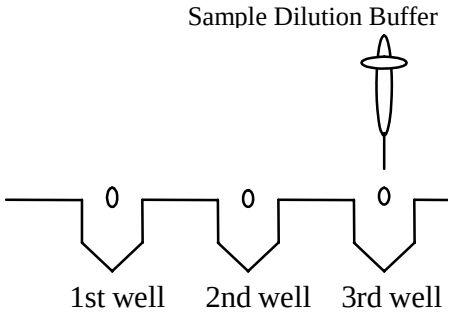
3. **Operation**

   (1) **Screening test** (refer to Table 1)

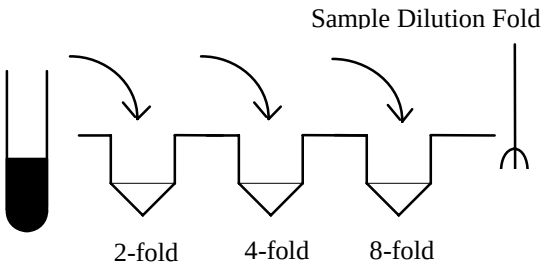
   1) Use a rigid or permanent type microplate with V-shaped wells (with mesh).
   One sample uses 3 wells.



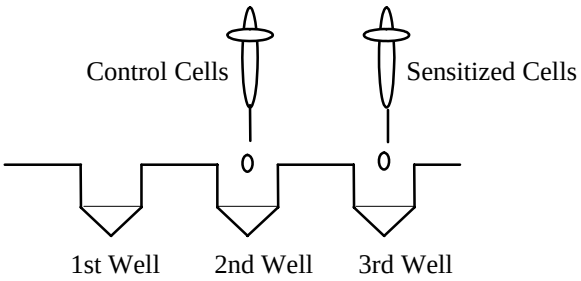
   2) Dispense by a dropper 25 µL of the sample dilution buffer in the first through to the 3rd well.



3) Dispense in the first well by a diluter or micropipette 25 µL of a sample and serially dilute by two-fold dilution up to the 3rd well.



4) Thoroughly suspend the sensitized and control cells prepared in advance, then dispense by a dropper 25 µL each of the control and sensitized cells in the 2nd well and 3rd well, respectively. Separate droppers must be used for the control and sensitized cells. Do not dispense any cells in the first well.



5) After thoroughly mixing the microplate contents on a microplate mixer, leave the microplate to stand for one hour on a vibration free table at the room temperature (15 ~ 30°C) and determine. After dispensing the sensitized and control cells, make sure to mix them with the sample dilution buffer as quickly as possible. If they are let to precipitate without mixing with the sample dilution buffer, proper hemagglutination patterns may not be obtained.

Table 1: Screening test procedures

Well No.		1	2	3
Sample dilution buffer	(µL)	25 ↘	25 ↘	25 ↗
Sample	(µL)	25	25	25
Sample dilution		1 : 2	1 : 4	1 : 8
Sensitized cells	(µL)	-	-	25
Control cells	(µL)	-	25	-
Thoroughly mix cells and samples and leave to stand for one hour at the room temperature for determination				

Determination of hemagglutination patterns

Determination of hemagglutination patterns is made according to the following criteria.

	Hemagglutination Patterns	Determination (Hemagglutination)
	All cells precipitate at the center of a well.	Hemagglutination (-)
	Most cells precipitate at the center of a well.	
	Cells partly precipitate at the center of a well and partly hemagglutinate around the precipitation like mesh.	Hemagglutination (+)
	Size of precipitation in the center of a well becomes smaller and mesh-like hemagglutination becomes clear.	
	Cells clearly hemagglutinate like mesh across a well.	

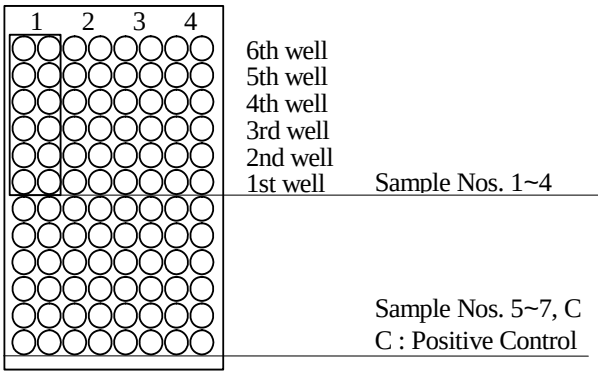
Determination by the screening test

Samples with negative (-) hemagglutination of the sensitized cells are determined anti-HBs negative. Samples with negative (-) hemagglutination of the control cells but with positive (+) hemagglutination of the sensitized cells are determined anti-HBs positive. Samples with positive (+) hemagglutination of both sensitized and control cells are subjected to a confirmatory test for determination.

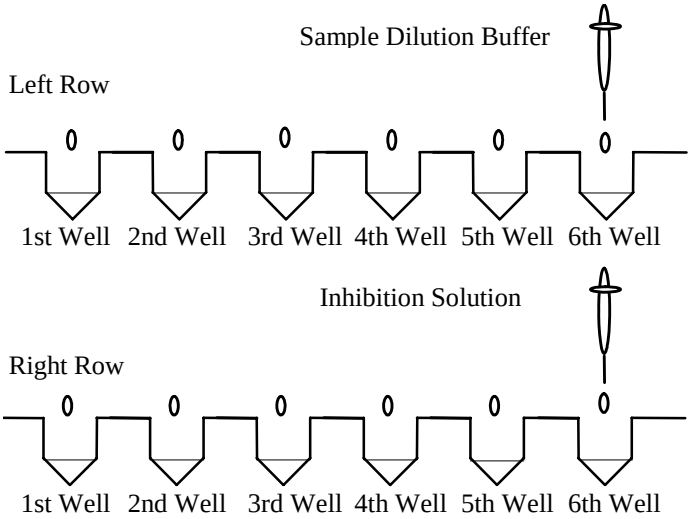
	Negative	Negative	Positive	Suspended (to Confirmatory Test)
3rd Well (Sensitized cells)	◉	◉	●	●
2nd Well (Control cells)	◉	●	◉	●

(2) **Confirmatory test** (Refer to Table 2)

1) Use a rigid or permanent type microplate with V-shaped wells (with mesh). Use two rows of 6 wells per sample.



2) Dispense by a dropper 25 µL each of the sample dilution buffer in 6 wells on the left row and by another dropper 25 µL each of the inhibition solution in 6 wells on the right row.



3) Dispense by a micropipette or diluter in the first well in both right and left rows 25 µL each of a sample and serially two-fold dilute up to the 6th well.

4) Dispense by a dropper one drop (25 µL) of the sensitized cells in all wells.

5) Thoroughly mix the well contents on a microplate mixer, cover the microplate with a cover and leave it to stand for one hour on a vibration free table at the room temperature (15 ~ 30°C) for determination.

Table 2: Confirmatory test procedures

Well No.		1	2	3	4	5	6
Sample dilution buffer	(µL)	25	25	25	25	25	25
Sample	(µL)	25	25	25	25	25	25
Inhibition solution	(µL)	25	25	25	25	25	25
Sample	(µL)	25	25	25	25	25	25
Sample dilution		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
Sensitized cells	(µL)	25	25	25	25	25	25
Thoroughly mix the well contents and leave to stand for one hour at the room temperature on a vibration free table for determination.							

Determination by the confirmatory test

1) When a sample diluted with the sample dilution buffer (left row) and the inhibition solution shows the difference in hemagglutination titers (reduction of hemagglutination titer by HBsAg) of 2 wells or more, it is determined positive.
2) When a sample diluted with the sample dilution buffer and inhibition solution does not show difference in hemagglutination from the 1st well through the 5th well, it is determined negative.
3) When hemagglutination difference is other than the above, the following applies.

	Sample Dilution Fold						Determination
	2-fold	4-fold	8-fold	16-fold	32-fold	64-fold	
Sample side	●	●	●	●	●	●	Suspended. Sample of high titer is suspected. Test again at higher dilution.
Inhibition side	●	●	●	●	●	◉	
Sample side	●	●	●	●	●	●	
Inhibition side	●	●	●	●	●	●	

(3) **Quantitative test**

By determining the end titer, this kit can quantitatively determine samples tested positive by the screening or confirmatory test.

1) Dispense one drop (25 µL) each of the sample dilution buffer in 8 wells in two rows.
2) Dispense by diluters or micropipettes 25 µL each of the positive control and a sample in the first wells and serially dilute them up to the 8th well by 2-fold dilution.
3) Dispense by a dropper 25 µL of the sensitized cells in all wells.
4) After mixing the well contents on a microplate mixer, cover the microplate with a cover and leave it to stand for one hour at the room temperature and determine. (The maximum dilution at which samples hemagglutinate is determined to be their anti-HBs titer.)

Assay precautions

1. **Collection of samples and their handling**
   1) Collect sera or plasma in the normal way and avoid their hemolysis. Inactivation of sera does not practically affect the assay results.
   2) If samples are not assayed immediately after their collection, store them in a refrigerator (after adding 0.1% final concentration of sodium azide) or in a freezer.

2. **Interfering substances**
   1) Red blood cells or fibrin adversely affect determination and should be removed.
   2) Up to 2000 FTU of chylomicron, 20 mg/dL of bilirubin C, 20 mg/dL of bilirubin F and 500 mg/dL of hemoglobin do not adversely affect the determination.

Specifications

1. **Sensitivity**

   In the qualitative test using the positive control as a sample, this kit shows hemagglutination up to 32 to 128-fold dilution.

2. **Specificity**

   This kit tests negative the anti-HBs negative panel sera and tests positive the anti-HBs positive panel sera.

3. **Reproducibility**

   When the positive control is tested 10 times as a sample, this kit shows hemagglutination within ± 1 well of the mode.

Correlation

Correlation of this kit and a kit of the same assay principle manufactured by Company A was studied.

(Result) 70 samples tested showed 100% correlation.

This kit \ A Company	-	+
-	30	0
+	0	40

Handling cautions

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. **Cautions in assays**
   1) Check accuracy of tools and properly use them according to their instructions.
   2) When using the droppers supplied with this kit, wipe off solution on the tip and slowly dispense at a given speed by holding them upright.
   3) After dispensing the sensitized and control cells, thoroughly mix the well contents on a microplate mixer with minimum loss of time.
   4) To prevent the well contents from evaporation, cover the microplate with a plastic plate, etc. while waiting for their reaction.
   5) When frequently used microplates are used, hemagglutination pattern may become poor due to insufficient washing and/or scratches on the well surface.

2. **Cautions in the use of this kit**
   1) Do not use expired reagents.
   2) Do not use reagents of different production lots.
   3) Leave to stand the sensitized and control cells for 1 hour at the room temperature (2 ~ 30^{o}C) or overnight at 2 ~ 8^{o}C before use.
   4) All reagents must be returned to the room temperature (15 ~ 30^{o}C) before use.
   5) All samples should be handled carefully as if they were capable of transmitting HBV, HCV or HIV.

6) The positive control is not inactivated (heat treatment at 60°C for 10 hours) and should be handled carefully. Although it is tested negative for HBV, HCV antibody and HIV antibody, it should be handled like samples as if it were capable of transmitting these viruses.

3. **Cautions after use of this kit**

1) Reagents contains a preservative (0.1% sodium azide) but once they are opened, they should be handled carefully to avoid contamination with microorganisms. Once they are contaminated, it will take a longer time for the sensitized and control cells to precipitate or determination may become difficult. To prevent formation of explosive metal azide by sodium azide, drain them down with a sufficient volume of water.

2) Microplates used in the assay should be immersed overnight in 1% (W/W) sodium hypochlorite, washed with water using a microplate washer, immersed overnight in detergent, washed again with water, and dried.

3) Diluters used in the assay should be sterilized in 2% glutaraldehyde for one hour, rinsed and burnt by gas burner for 5 to 10 seconds.

4) It is desirable that other tools are sterilized in the above manners or autoclaved (at 121°C for more than 20 min).

4. **Cautions in storage**

1) The sensitized and control cells are stable for one month after preparation if stored at 2 ~ 8°C.

2) A small quantity of insoluble substance formed in the suspension solution, sample dilution buffer and inhibition solution does not adversely affect the hemagglutination reaction.

5. **Others**

1) Vials and tools contained in this kit should not be used for other purposes.

2) Overall diagnosis should be made not only by the results of this assay but by taking into consideration clinical findings and the results of other tests.

Storage and shelf life

Store at 2 ~ 8°C. (Do not freeze.)

This kit is stable for one year after the date of manufacture and must be used up before the expiry date.

Packages

1 kit	for 100 tests	Code No. 1AB1
	for 200 tests	Code No. 1AB2
	for 1000 tests	Code No. 1AB3

Reference

Blumberg BS, Altec HJ, Visnich S: A new antigen in leukemia sera. JAMA **191**: 541–546, 1965.

Institute of Immunology Co., Ltd.
1-1-10, Koraku, Bunkyo-Ku,
Tokyo 112-0004, JAPAN
Tel: +81-3-3814-4081
Fax: +81-3-3814-5957