PREZYME "SEIKEN"

INTENDED USE

PREZYME "SEIKEN" EIA reagents are intended for the in vitro detection and quantitative determination of immunoglobulin antibody to infectious diseases of laboratory animals such as Sendai virus (HVJ), Murine hepatitis virus (MHV), Mycoplasma pulmonis and Tyzzer's organism (Bacillus piliformis).

INTRODUCTION

Recent biological tests using laboratory animals in the medical science and biochemistry fields must meet increasing demands for precision. Genetic control of laboratory animals and environmental control of their breeding conditions are two important factors to attain enough precision and reproducibility of the test.

Physical and chemical environmental factors are very important, and these are relatively easy to control and are usually paid proper attention to in general laboratories. However, biological factors, which are no less important than the former, are much more difficult to control and negligence in this area will potentially ruin animal experiments.

Infection of laboratory animals with bacteria and parasites are serious obstructions to the constancy of test conditions in tests of this nature. To prevent such contamination is equally important to the genetic control of the animals.

Once contamination of the animal breeding unit in a laboratory spreads unchecked, it will cause devastating damages to the animals as well as the experiments themselves using these animals. People who are working in the contaminated area may also be exposed to risks if the organism is cross-infectious.

Detecting the contamination as early as possible is therefore essential to contain the infection before it spreads further and the laboratory becomes grossly contaminated with the pathogen.

Isolation of the organism, histopathological and serological tests are currently used for monitoring animals. Among these, the serological test has advantages over the other test due to its specificity and the persistence of the specific antibody in the sera of infected animals.

The complement fixation test, agglutination test and immunofluorescent test are all used to detect specific antibodies to pathogens and are characterized by their ability to detect infections early, their simple procedures, their low potential to contaminate the environment with pathogens, and low costs. Because of these advantages, the conventional antibody tests are widely used.

However, enzymeimmunoassay to detect antibodies has become commercially available and is becoming more popular as it is possible to test a lot of specimens in a run, and its sensitivity and specificity are usually far superior to other tests.

PREZYME "SEIKEN" EIA reagents were formulated for the detection and quantification of antibodies to the infectious diseases of laboratory animals. Microtiter plates coated with specific antigen of the pathogens are currently available for the following organisms; Sendai virus (HVJ), Murine hepatitis virus (MHV), Mycoplasma pulmonis and Tyzzer's organism. The separate strip format used for the coated plate enables laboratories to test a small number of speciments at a time.

Sendai virus is a RNA virus belonging to the paramyxovirus group which infects mice and rats with high frequency, causing usually respiratory disorders. The infection may often end without the animal developing any apparent symptoms. Detection of antibody to this organism is regarded as an important measure to find the infection.

Murine hepatitis virus is a RNA virus belonging to the corona virus group and infects mice causing hepatitis, which remains inapparent. Blood antibody detection is the preferred method of detecting the infection.

Sialodacryoadenitis (SDA) virus is RNA virus belonging to corona virus group. This infects rats causing acute sialodacryoadenitis. The virus also shares some common antigenicity with MHV, and so the antibody to this virus may react with the MHV antigen coating the plate of our PREZYME test. The elevation of SDA antibody in rat is generally more conspicuous than that of MHV antibody in mouse.

Mycoplasma pulmonis causes mainly respiratory diseases in mice and rats infected with this organism, most cases do not develop any apparent symptoms. However, double infection of the animals with the Mycoplasma and other viruses or bacteria induces Mycoplasma to produce symptoms. Serodiagnosis of this infection is as useful as antigen detection from the upper respiratory tract.

Tyzzer's organism infects mice and rats causing tache blanche focal necrosis of the liver and severe hemorrhagic enteronitis. Direct detection of Tyzzer's organism in the herd has been recognized as the most definite means of confirming its presence. However the infection quite often remains inapparent and serodiagnosis is also considered to be an efficient detection method.

PREZYME EIA reagents enable laboratories to carry out serodiagnosis of these infections using small volume serum specimens (10 ul of 10-fold diluted serum). The complement inactivation required for Complement fixation is not necessary for this test. The separate strip format of the microtiter plate wells allows flexibility in the test. A small number of samples can be checked economically in a run, and an appropriate combination of wells, targeted at different organisms, can be checked in a given run. The test can be completed in about 3 hours and the results can be read visually. The entire test is performed at room temperature (15—30°C).

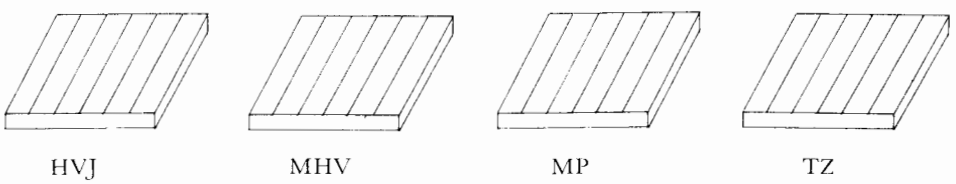
PRINCIPLE

This method is an enzyme immunoassay using antigen-coated microtier plates. When a specimen is added to the antigen coated wells, the specific antibody will bind to the antigen. This antigen/antibody complex can then be detected by the addition of an enzyme-labelled antispecies antibody and substrate. The enzyme activity is proportional to the quantity of antibody present in the specimen. The results can be read visually and also by microplate spectrophotometer.

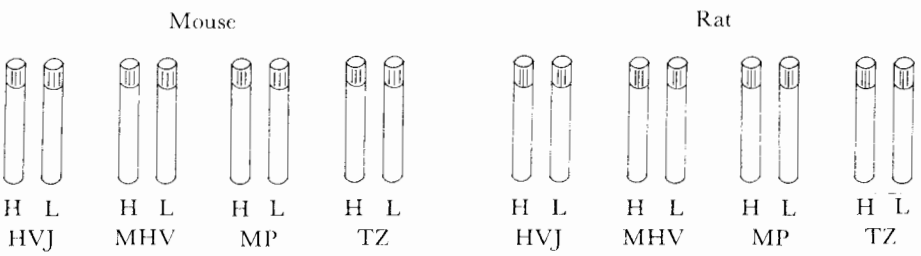
PRODUCTS COMPOSITION

The reagent kits must be used in combination with each other, as the antigen-coated microtiter plates, positive control sera and color development reagents are all available from us, separately. You can choose these products according to your requirements and use them in combination with each other.

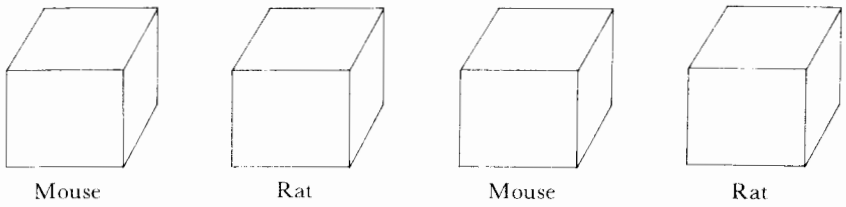
Antigen-coated Microtiter plate



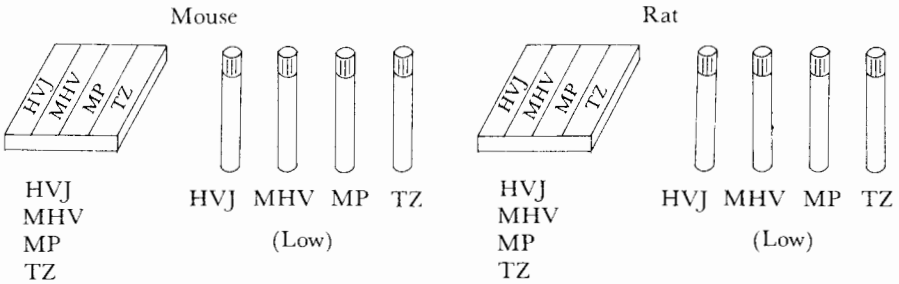
Positive control (High and Low)



Color development reagent



PREZYME 4D Antigen-coated Microtiter plate + Positive control (Low)



COLOR DEVELOPMENT REAGENT (Mouse)

COLOR DEVELOPMENT REAGENT (Rat)

Components of the Reagent Kit

1. Diluent

Phosphate-buffered saline containing bovine serum albumin.

2. Enzyme-labelled antibody

Lyophilized peroxidase-labelled anti-mouse antibody prepared in rabbit.
Lyophilized peroxidase-labelled anti-rat antibody prepared in rabbit.

3. Substrate A

Lyophilized o-phenylene diamine and phosphate-buffered saline.

4. Substrate B

Citrate-phosphate-buffered saline containing 0.02% hydrogen peroxide.

5. Stock washing solution

(10 × concentrated)
10 × concentrated solution of phosphate-buffered saline containing Tween 20.

6. Stop solution

1.5N Sulfuric acid.

Reagent	For 1 plate (96 wells)	For 4D (64 wells)
Diluent	13ml × 1	13ml × 1
Enzyme-labelled antibody	2ml × 6	2ml × 4
Substrate A	2ml × 6	2ml × 4
Substrate B	15ml × 1	15ml × 1
Stock washing solution (10x concentrated)	50ml × 1	50ml × 1
Stop solution	13ml × 1	13ml × 1

Storage and Shelf Life

Store at 2 to 10°C
Shelf life is 1 year after manufacture.

ANTIGEN-COATED MICROTITER PLATE

HVJ-Antigen Plate (Sendai virus antigen-coated microtiter plate)
MHV-Antigen Plate (Murine hepatitis virus antigen-coated microtiter plate)
MP-Antigen Plate (Mycoplasma pulmonis antigen-coated microtiter plate)
TZ-Antigen Plate (Tyzzer's organism antigen-coated microtiter plate)

The wells are coated with purified inactivated antigen and also contain a preservative solution.

Name of strain used

Sendai virus	MN strain
Murine hepatitis virus	NuU strain
Mycoplasma pulmonis	FuM-925T strain
Tyzzer's organism	RT strain

The plates used in the kit are the divided type microtiter plate (96 wells:16 wells/strip × 6 strips) (PREZYME 4D : each item 16 wells/strip × 1)
1 plate frame is also included.

Storage and Shelf Life

Do not freeze. Store at 2 to 10°C
Shelf life is 1 year after manufacture.

POSITIVE CONTROL SERA (HIGH and LOW)

HVJ-Positive Control (Mouse) (Sendai virus control serum)
MHV-Positive Control (Mouse) (Murine hepatitis virus control serum)
MP-Positive Control (Mouse) (Mycoplasma pulmonis control serum)
TZ-Positive Control (Mouse) (Tyzzer's organism control serum)

HVJ-Positive Control (Rat) (Sendai virus control serum)
MHV-Positive Control (Rat) (Murine hepatitis virus control serum)
MP-Positive Control (Rat) (Mycoplasma pulmonis control serum)
TZ-Positive Control (Rat) (Tyzzer's organism control serum)

These positive control sera were obtained by immunizing mice/rats with the appropriate microganism adjusted to a required titer.
Each kit has two positive controls-high and low.
PREZYME 4D includes 4 low positive controls (one for each item).

Storage and Shelf Life

Store at 2 to 10°C.
Shelf life is 1 year after manufacture.

Use Instructions and Precautions

1. Do not combine or mix reagents from different manufacturing lots.
2. Ensure that the positive control solution is incubated on every run.
3. The time of incubation should be exactly as specified.
4. Add the reagents quickly so as not to allow the wells to dry up.
5. Do not freeze the microtiter plates. Store them at 2 to 10°C.
6. Take care not to damage the external and internal surfaces of the wells.
7. Avoid direct sunlight while carrying out the test.
8. To prevent cross-contamination use 1 tip per specimen.
9. The diluent may be used even if it is opaque.
10. Reconstitute the substrate solution immediately before use, then use it within 1 hour.
11. When preparing the substrate solution, avoid contact with metals.
12. Take care to prevent the substrate and the stop solutions from coming in contact with the body or clothing.

Test Procedure

1. Instruments

1) Micropipette (Preferably, Gilson P20 and P200)
2) Measuring pipette
3) Micro test tube
4) Measuring cylinder
5) Erlenmyer flask
6) Microtiter plate mixer

2. Preparation of Reagents

Reagent	Preparation method For 1 plate (96 wells) or for 4D (64 wells)	Prepared reagent	Storage period after preparation
Diluent	Ready to use		1 year when stored at 2 to 10°C
Stock washing solution (10 × concentrated)	Pipet out an amount of stock solution according to the number of specimens, and dilute it 10-fold with purified water.	Washing solution	1 month when stored at 2 to 10°C
Enzyme-labelled antibody	Prepare appropriate number of vials according to the number of specimens. Add 2ml of washing solution to a vial.	Enzyme-labelled antibody solution	1 month when stored at −20 to −30°C (freeze and thaw only once)
Substrate A Substrate B	Prepare appropriate number of vials immediately before use according to the number of specimens. Add 2ml of substrate B to a vial of substrate A.	Substrate solution	Use within 1 hour.
Stop solution	Ready to use.		1 year when stored at 2 to 10°C

3. Other reagents required for the test but not provided in this kit (available separately)

Antigen-coated plate and positive control — Refer to the last page.

4. Dilution of specimens

Dilute the specimens 10-fold with physiological saline. (If they are already diluted at the time of bleeding, further dilution is not necessary.)

Positive controls are already diluted and are ready for use.

5. Screening Test

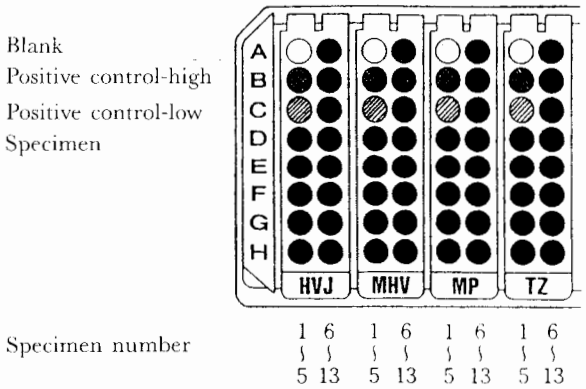
1) Preparation of test plates

① Select the number of strips needed for the test. (1 blank, 1 high positive and 1 low positive control should be included in each test.)
② Remove the strips by cutting along the grooves, and fix them on to the frame provided.
③ Remove the seal and label the strips.

2) Addition of specimens and positive controls

① Discard the preservative solution (Note 1)
② Add 90μℓ of diluent to all the wells except the blank. To the blank add 100μℓ of diluent.
③ Add 10μℓ of control sera (High and Low) and diluted specimens to the appropriate wells. (Final dilution of serum becomes 100-fold.)

Proceed with this step quickly.



○	Diluent	100μℓ		
◉	Diluent	90μℓ	Positive control High	10μℓ
▨	Diluent	90μℓ	Positive control Low	10μℓ
●	Diluent	90μℓ	Diluted specimen	10μℓ

(Final dilution of serum, 100-fold)

④ Mix for 15 to 30 seconds on a microtiter plate mixer. (Note 2)
⑤ Cover the plate and incubate at room temperature (15 to 30°C) for 1 hour.
⑥ Discard the contents of the wells and wash the plate 3 times. (Note 3)

3) Additional of the enzyme-labelled antibody solution

① Add 100μℓ of the enzyme-labelled antibody solution (anti-mouse antibody for mouse specimens and anti-rat antibody for rat specimens) to all the wells including the blank and mix for about 15 seconds.
② Cover the plate and incubate at room temperature (15 to 30°C) for 1 hour.
③ Discard the contents of the wells and wash the plate 3 times. (Note 3)

4) Addition of substrate solution

① Add 100μℓ of substrate solution prepared immediately before use to all the wells and mix for about 15 seconds.
② Cover the plate and incubate at room temperature (15 to 30°C) in the dark for 30 minutes. (Note 4)

5) Addition of stop solution

Add 100μℓ of stop solution to all the wells in the same sequence and at the same intervals as the substrate solutions and mix for about 15 seconds.

6) Results

① Read the results within 1 hour either by visual inspection by the measurement of optical density (OD at 492nm) with a microplate-spectrophotometer.

② Confirm that the blank wells are almost colorless, that the high positive control wells are dark reddish brown and that the low positive control wells are light reddish brown.

③ If the plates are read visually, compare the color of specimen wells with that of the low positive well.
Color of specimen well ≧ Color of low positive well — Antibody positive
Color of specimen well < Color of low positive well — Antibody negative

④ If a microplate-spectrophotometer is used, compare the OD of specimen wells with that of the low positive well.
OD of specimen well ≧ OD of low positive well — Antibody positive
OD of specimen well < OD of low positive well — Antibody negative

6. Quantitative Assay

The same kit can be used for the quantitative assay of antibody. In this case, use 2-fold dilutions starting with 1 in 10.
The test procedure is the same as for the screening test.
The reciprocal of the last dilution giving a positive result is taken as the antibody titer.
The significance of a positive result will have to be decided after taking into consideration factors such as, the clinical picture, time of specimen collection etc.

Note 1: Discarding of preservative solution

Discard the preservative solution in wells quickly, by holding the convexity of the lower central frame of the microtiter plate (area showing numericals 5,6,7 and 8) from both sides of the frame firmly, and turning the microtiter plate upside down. Alternatively, aspirate the solution using an aspirator.

Note 2: Mixing

After the addition of specimen to the microtiter plate wells, mix thoroughly for 15 to 30 seconds on the microtiter plate mixer. If it is mixed incompletely, variations in the intensity of color development may result.

Note 3: Washing

Add about 200$\mu\ell$ of washing solution to all the wells, shake for several seconds on the microtiter plate mixer, and discard the washing solution either by inverting the plate or by aspirating it with an aspirator. Finally, gently tap the microtiter plate on clean paper towel to remove the washing solution completely from the wells.

Note 4: Enzymatic reaction

Cover with aluminum foil or with an empty, colored plastic box or keep inside a drawer.

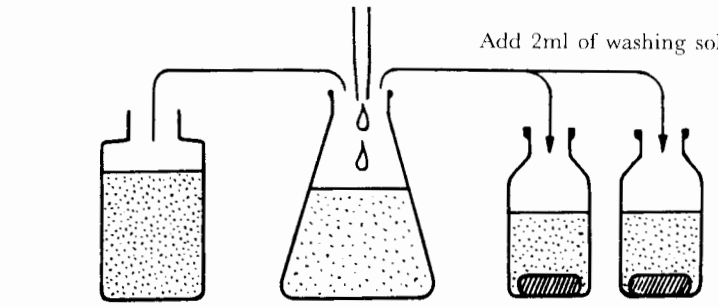
References:

1) Voller.A.et al.: Enzyme immunoassays in diagnostic medicine, Bull. Wld. Hlth. Org., 53:55 (1976).
2) Fujiwara, K.et al.: Infectious disease in experimental animals. Soft science, Tokyo (1977).
3) Iwai, H.,et al.: Immunoglobulin Classes of Anti-Sendai Virus Antibody Detected by ELISA in Infected Nude House Serum, Microbiol. Immunol., 28(4). 481(1984).
4) Matsubara, J., et al.:Serodiagnosis of Mycoplasma pulmonis Infection in Mice and Rats by an Enzyme-linked Immunosorbent Assay, Exp.Anim.,34(1),49(1985)
5) Toriumi, W.,et al.: Application of Enzyme-Linked Immunosorbent Assay (ELISA) to Detection of Antibodies against Tyzzer's Organism (Bacillus piliformis) in Mice, Jpn. J. Vet. Sci.,48(6),1241 (1986).

Preparation of Reagents

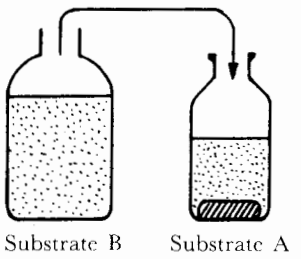
Dilute 10-fold with purified water.

Add 2ml of washing solution to a vial.



Washing of microtiter plates

Add 2ml of substrate B to a vial immediately before use.

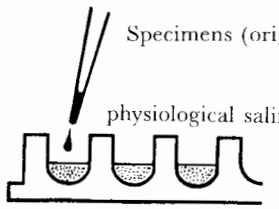


Substrate solution

Use within 1 hour after preparation.

Diluent and stop solution are ready for use.

Dilution of specimens

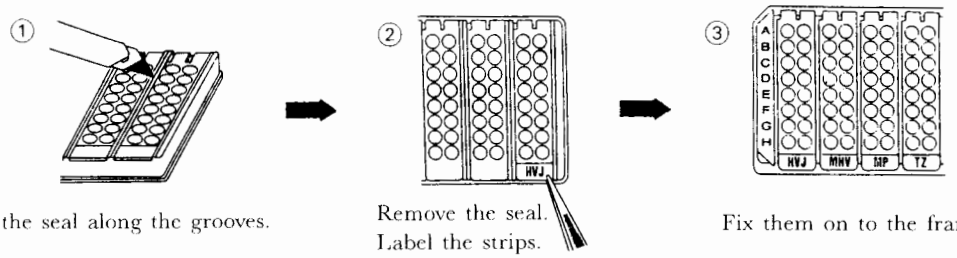


(Required volume is 10μℓ per specimen.)

Specimen number 1 2 3

Screening test

Preparation of antigen plates

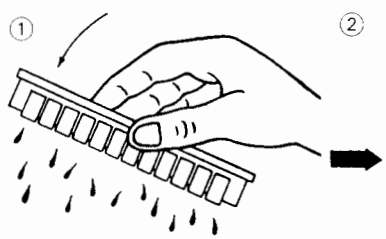


Cut the seal along the grooves.

Remove the seal.
Label the strips.

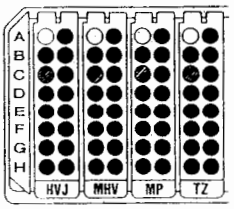
Fix them on to the frame provided.

Addition of specimens and positive control



Discard the preservative solution in the microtiter plate. (Note 1)

② Blank.
Positive control-High.
Positive control-Low.
Specimen

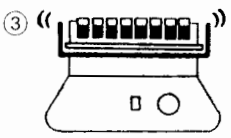


Specimen number

○	Diluent	100μℓ		
●	Diluent	90μℓ	Positive control High	10μℓ
▨	Diluent	90μℓ	Positive control Low	10μℓ
●	Diluent	90μℓ	Diluted specimen	10μℓ

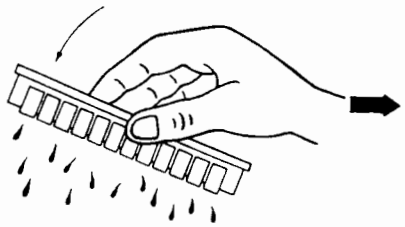
(Final dilution of serum, 100-fold)

Addition of specimens and positive control.

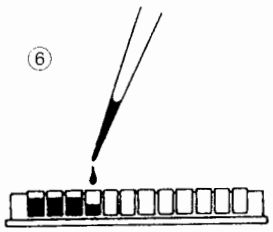


Mix for 15 to 30 seconds. (Note 2)

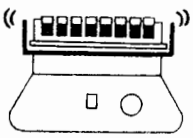
After covering the plate incubate at 15 to 30°C for 1 hour.



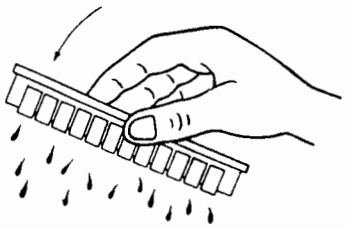
Discard liquid in the wells.



Add about 200μℓ of washing solution to all the wells.

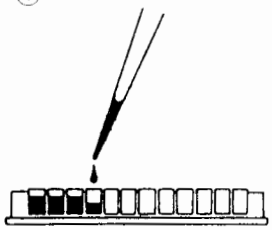


Mix for several seconds.

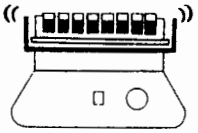


Discard washing solution.
Repeat 3 times ⑥ to ⑧ (Note 3)

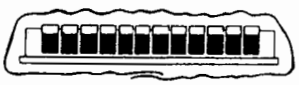
Addition of the enzyme-labelled antibody solution



Add 100μℓ of the enzyme-labelled antibody solution to all the wells.

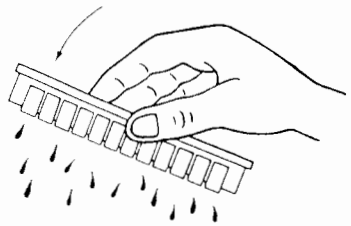


Mix for about 15 seconds.



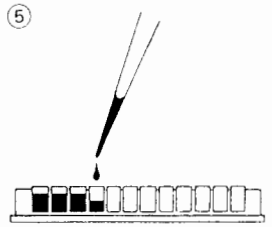
After covering the plate incubate at 15 to 30°C for 1 hour.

④



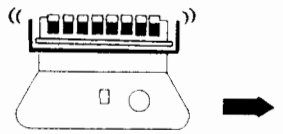
Discard liquid in the wells.

⑤



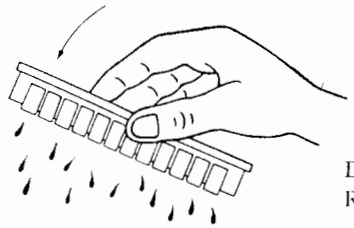
Add about 200μℓ of washing solution to all the wells.

⑥



Mix for several seconds.

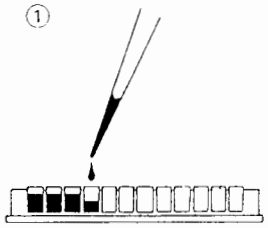
⑦



Discard washing solution.
Repeat 3 times ⑤ to ⑦ (Note 3)

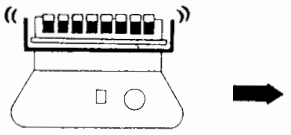
Addition of substrate solution. (Proceed with this step quickly)

①



Add 100μℓ of the substrate solution to all the wells.

②



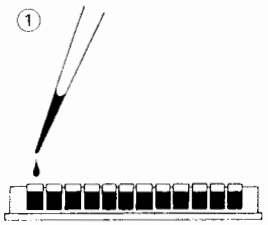
Mix for about 15 seconds.

③

After covering the plate incubate at 15 to 30°C for 30 minutes in the dark. (Note 4)

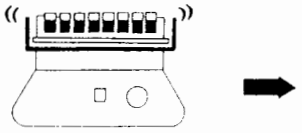
Addition of stop solution

①



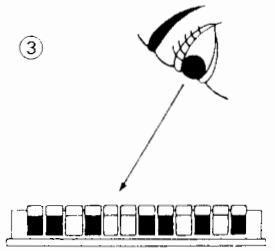
Add 100μℓ of stop solution to all the wells.

②



Mix for about 15 seconds.

③



Read the results either by visual inspection or by measurement of optical density (OD at 492nm) with a microplate-spectrophotometer within 1 hour.

Results

If the plates are read visually, compare the color of specimen wells with that of the low positive well.

Color of specimen well ≧ Color of low positive well — Antibody positive
Color of specimen well < Color of low positive well — Antibody negative

If a microplate-spectrophotometer is used, compare the OD of specimen wells with that of the low positive well.

OD of specimen well ≧ OD of low positive well — Antibody positive
OD of specimen well < OD of low positive well — Antibody negative