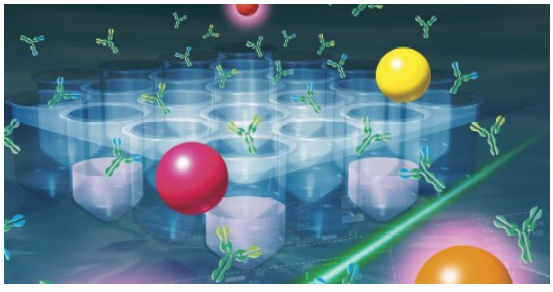
WAKFlow® HLA Antibody

Class Ⅰ（MR） & Class Ⅱ（MR）

Operating Manual

Research use only

—1st Edition—



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Wakunaga Pharmaceutical Co., Ltd.

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Flowsheet: *WAKFlow*® **HLA Antibody Class Ⅰ（MR）&Class Ⅱ（MR）**
- Principles and Outline of Procedure -

1. Principles of Measurement

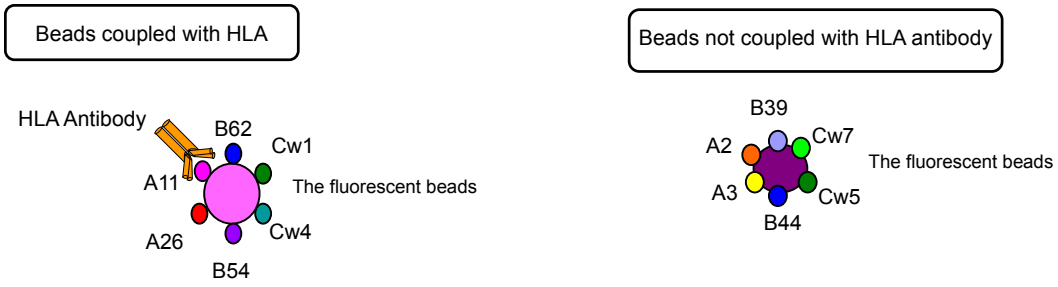
WAKFlow® **HLA Antibody Class Ⅰ（MR） or** *WAKFlow*® **HLA Antibody Class Ⅱ（MR）** is a reagent for research use only to detect and identify an antibody (HLA antibody) against the HLA Class I molecule or the HLA Class II molecule in human serum. This product is used to detect antibodies, using the xMAP technology of Luminex Corporation.

The operating procedures consist of the following 2 steps.

Ⅰ）Incubation of beads with the sample serum

Incubate a sample serum with the fluorescent beads that are pre-coated with HLA Class I or Class II molecules (extracted and purified from human B cell-derived cell lines).

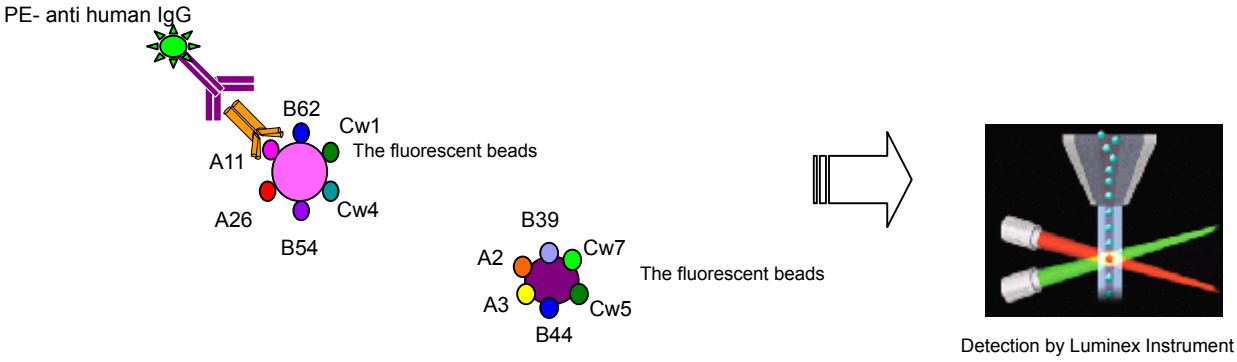
If HLA antibody exists in the sample serum, it will bind to the beads that are coated with HLA Class I or Class II molecules, to which the antibody was reactive (including Cross Reactivity Groups (CREG)). The figures below show the case of Class I.



Ⅱ） Labeling by anti- Human IgG conjugated to phycoerythrin and Analysis of alloantibody specificity

The sensitized beads washed to remove unbound alloantibody are labeled with anti-Human IgG conjugated to phycoerythrin.

As each of these fluorescent beads is differently dyed with a fluorescent substance, fluorescent signals by the labeled antibody can be obtained from the fluorescent beads besides the beads-derived fluorescent signals. In other words, the kinds (colors) of fluorescent beads and a fluorescent signal from a labeled antibody are simultaneously identified and detected. In addition, the specificity of a HLA antibody can be determined from the pattern of a fluorescent signal.



2. Materials Provided

This product consists of the following reagents.

These reagents in the Kit should not be used in combination with the product of a different, lot number or any other kit reagent of a different product.

25 test／kit

① Beads mix ····· 1 tube
② 10× washing solution ····· 1 tube
③ Labeled antibody ····· 1 tube

Notes

1) All the reagents should be stored at 2 - 8ºC.

2) The beads mix (1) and the labeled antibody (3) should be stored under a light-shielded condition.

3. Procedure

3. 1 Cautions in operating procedure

1. All the procedures should be conducted with care under a as much darkened condition as possible.
2. Due to possible existence of infectious substances such as viruses, bacteria, etc. in the sample serum, care must be taken during the procedure for prevention of infection. (To be mentioned later)

3．2 Required instrument and equipment, but not provided

（1）For incubation of beads mix with a sample serum and the labeling reaction by a labeled antibody

・96-well V-shaped bottom plate

■Recommended product:

96-well V-shaped bottom microtiter plate（Nunc #442587）

・Micropipette (variable type: 1-20μL, 10-200μL, 100-1000μL) with appropriate pipette tips

・Multichannel pipette (if available, it is convenient)

・Continuously adjustable repetitive pipette (if available, it is convenient)

・Vortex mixer

・Plate mixer

・Microplate centrifuge (usable at 1,300 x g)

・Plate seal

Micropipette

Multichannel pipette

Continuously adjustable repetitive pipette

Vortex mixer

Plate mixer

Microplate centrifuge

（2）Detection and analysis

・Luminex Instrument and X-Y platform

・Personal computer

Luminex Instrument

3．3 Directions for use

3.3.1 Preparation

（1） Pre-treatment of a sample serum

Prior to the assay, do not fail to centrifuge the sample serum for 2 minutes at 10,000 x g and precipitate the insoluble substances.

Note: Contamination with any insoluble substances at the time of conducting the assay may lead to incorrect results due to the interference of the antigen-antibody reaction.

（2）Beads treatment

Stir thoroughly ① **beads mix** by a vortex mixer.

（3）Preparation of washing solution

1）When deposits are found in ② **10× washing solution**, heat the solution at below 37°C to dissolve, and ensure that deposits have been completely dissolved.

2）Transfer 5 mL of ② **10× washing solution** into a vessel and add 45 mL of purified water to prepare 50 mL of the washing solution. Store the remaining, unused amount of the washing solution at 2-8°C.

（4）Luminex Instrument: Warm up for ready use.

3.3.2 Procedure

(1) Incubation of the beads with a sample serum

> ※ Put on the gloves for the procedures of 1) - 11) mentioned below to prevent infection.
>
> ※ Use a 96-well V-shaped bottom plate for the procedure.

1） Pipette 25 μL ① **beads mix**

into the 96-well V-shaped bottom plate.

2） To this mix, add 5 μL of the sample serum.

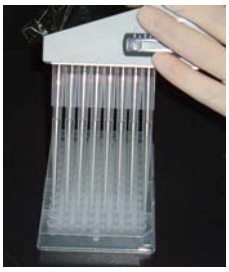
■ When a sample serum is taken, skim and put in the wells the supernatants with care not to take in the precipitations adhered to the wall of a tube or the bottom.

■ Change the pipette tip with a new one for each sample.

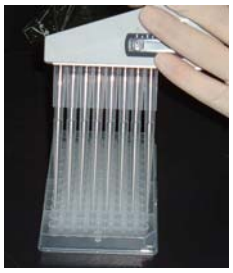
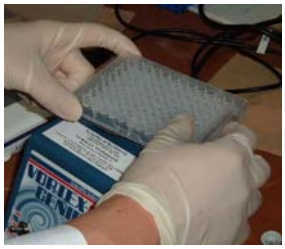
3） Seal tightly the plate so as not to allow a sample serum in a reaction well to mix with another one in the adjoining well, and then stir continuously at 25ºC for 30 minutes, using a plate mixer, under a light-shielded condition (in a light-protectable incubator or covered with aluminum foil, etc.).

■ During the reaction, set up Luminex Instrument.

4) After the reaction, add 150μL of the washing solution to each well, and centrifuge, using a plate centrifuge, for 2 minutes at 1,300 x g. After centrifugation, remove the supernatant by flicking or aspiration.



5) Once again, add 150μL of the washing solution in each well, and seal tightly. Stir thoroughly, using a vortex mixer, and centrifuge for 2 minutes at 1,300 x g. After centrifugation, remove the supernatant by flicking or aspiration

6) Repeat twice the procedure of 5).

■ During the centrifugation, dilute ③ **labeled antibody** by 100 times with the washing solution.

7) Add 50μL of the diluted, labeled antibody to each well.

8) After tight sealing, stir continuously at 25ºC for 30 minutes, using a plate mixer under a light-shielded condition (in a light-protectable incubator, or covered with aluminum foil, etc.).

9) Add 150μL of the washing solution to each well and centrifuge for 2 minutes at 1,300 x g. After centrifugation, remove the supernatant by snapping or aspiration.

10) Add 75μL of the washing solution to each well. If some beads mass is observed, seal the plate and disperse gently by a vortex mixer.

(2) Data Acquisition

Perform the measurement by using a template file that corresponds to the lot number of the beads mix based on the Luminex Instrument. For measurement, ensure that the temperature setting of the Luminex XYP has been in an “OFF” position.

■ If the samples are not ready for measurement, keep in a dark place.

＜**In case of the sample serum showing a non-specific reaction**＞

Some sample serum may show a non-specific reaction exceeding 1,000 fluorescence signals in all the beads. In such case, as there is a risk of distorting the analysis, repeat the test after eliminating the causative substances that produced the non-specific reaction in the sample serum by use of the serum treatment reagent (separately for sale) prior to the preparation

3．4 DATA Analysis

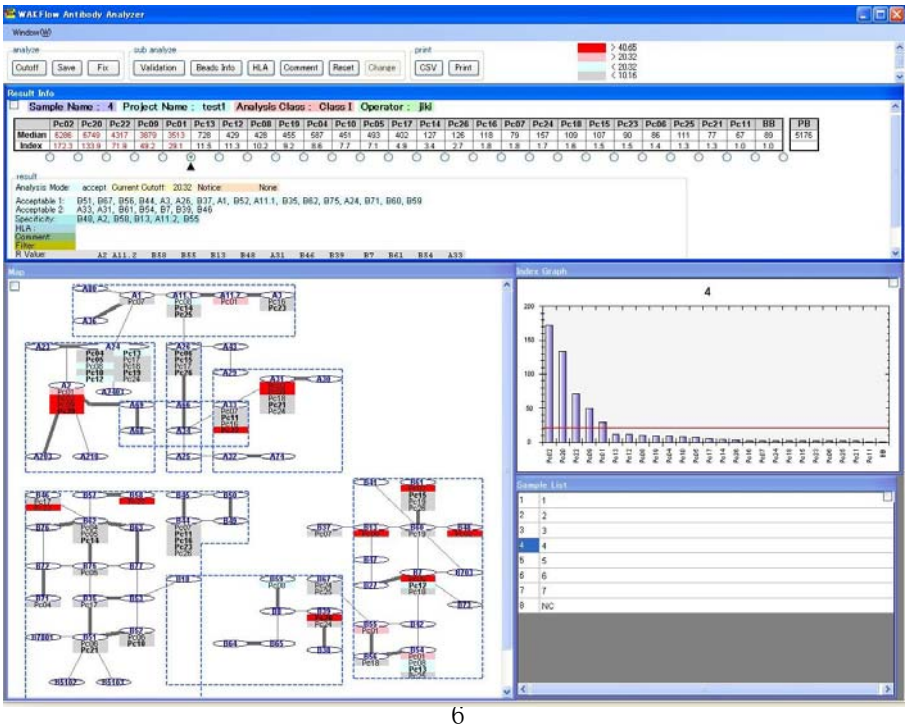
Analyze the CSV file containing the measurement results by "*WAKFlow*® **HLA Antibody Analysis Software**".

If the measurement has been carried out with the template-file not corresponding to a lot number of the beads mix, this software does not run a proper analysis. Due precaution must be taken.

The analysis is performed with the index values computed from the fluorescence signals (MFI: Median Fluorescence Intensity) of each bead, and then a pattern, either positive or negative, that is expressed to each bead, will determine a reactive specificity of HLA antibody.

"*WAKFlow*® **HLA Antibody Analysis Software**"

- This software provides an easy, visual judgment on the screen that has taken CREG (Cross Reactivity Group) into consideration. In addition, this software offers a convenient function for verifying the results such as the display of the kind of HLA type, R-values of the sample, etc.
- This software is compatible with Windows XP, Vista, but not supported with Macintosh OS.
- This software is given free of charge to the customers who have purchased our reagents.



4. Precautions on use/handling

1. General precautions

- This product is a reagent for research use only. Do not use for the treatment or diagnosis of a disease.
- Do not use any reagents that have been expired.
- Do not drink or lick any reagents. Take due care that the reagent may not stick to skin, or get into eyes or mouth. If the reagent accidentally sticks to skin, or gets into eyes or mouth, take emergency measures such as immediate, thorough washing with water. If something abnormal happens, consult a physician.

2. Precautions for users to prevent risks

(1) Viruses and bacteria

- Use the Kit only in designated area, taking into consideration that the sample serums may contain bacteria or other infectious substances.
- Use the pipette and tips to be employed only in a designated area.
- Wear a laboratory coat designated for the work, and use disposable, plastic gloves.
- After the work, clean the laboratory bench using 10% household bleach.
- In case that the laboratory bench is stained with serum, clean with 10% household bleach.

(2) Cautions on disposal of waste

After autoclaving dispose anything that came into contact with serum as waste, or dispose as medical waste. For the disposal method as medical waste, follow the disposal rule established for each laboratory facility.

3. Other Notifications

The specification of this product is subject to change without notice.

≪Storage≫ Store at 2 ~ 8ºC

≪Effective Period≫ 12 months (Expiry date is described on the outer package.)

≪Packing≫ 25 tests / kit

≪Contact address≫ **KOMABIOTECH**

URL: http://www.komabiotech.co.kr/

biotechnology
KOMABIOTECH

Manufactured by

Wakunaga Pharmaceutical Co., Ltd.

URL: http://www.wakunagahla.jp/english/index.html