For research use only

Kit for the determination of Mouse OVA specific IgE

DS Mouse IgE ELISA (OVA)

Contents of DS Mouse IgE ELISA (OVA)

Each kit (96 tests) contains the following reagents.

Standard A (lyophilized) ·····1 vial (For 0.5 mL)
Standard B (lyophilized) ·····1 vial (For 0.5 mL)
Standard C (lyophilized) ·····1 vial (For 0.5 mL)
Standard D (lyophilized) ·····1 vial (For 0.5 mL)
Standard E (lyophilized) ·····1 vial (For 0.5 mL)
Standard F (lyophilized) ·····1 vial (For 0.5 mL)
The concentration of each Standard is shown in the attached note.
Buffer ·····1 bottle (30 mL)
OVA-enzyme conjugate ·····1 bottle (15 mL)
HRP-labeled OVA (ovalbumin)
Antibody-coated wells ·····1 plate (96 wells)
Anti-Mouse IgE monoclonal antibody (rat)
Wash buffer concentrate ·····1 bottle (90 mL)
Substrate ·····1 bottle (15 mL)
Stop solution ·····1 bottle (15 mL)
Microplate for predispensing samples ·····1 plate (96 wells)

Assay method

1. Instruments and materials required

Pipettes with disposable tips: 15, 500µL, multichannel pipettes: 100, 110, 150µL, ELISA washer, Microplate reader equipped with 450 nm (as the main wave length) and 620 or 630 nm (as the reference wave length), others (cutter-knife, stop-watch, papertowel, aluminum foil, etc.).

2. Sample

Use mouse serum as a sample. Avoid repeated freezing and thawing of samples. In the case of samples containing a high concentration of Mouse IgE, dilute them with the Buffer in appropriate vessels prior to predispensing.

Ex. 5-fold dilution : 20µL of sample + 80µL of Buffer
10-fold dilution : 15µL of sample + 135µL of Buffer

3. Preparation of reagents

(1) Standard solutions
Accurately add 0.5 mL of purified water to Standard A-F vials. Stand for 15 min and then shake the vials gently to dissolve the contents thoroughly. In the case of storage, keep the vials closed and stored at 2-10°C, and use them within 1 week. For a long storage, keep them frozen. Just before usage, thaw them at room temperature gradually and mix well.

(2) Antibody-coated wells
Cut and remove the seal covering the needed number of Antibody-coated wells for samples and Standard solutions just before use. Decant the contents of each well, then tap the wells upside-down on a paper towel to remove the contents completely. Unused wells with the seal intact should be stored at 2-10°C.

(3) Wash buffer
Dilute the Wash buffer concentrate 10-fold with purified water. Use this as a Wash buffer. (This Wash buffer is stable for 1 week at 2-10°C.)

(4) Microplate for predispensing samples
Use this plate when Standard solutions and samples are predispensed prior to ELISA. A multichannel pipette is recommended for dispensing samples from this microplate into the Antibody-coated wells to avoid time differences among the assay wells.

(5) Substrate
Use the contents of the bottle without any dilution. Because the Substrate is readily colored due to contact with metal and/or exposure to light, keep it shielded from light and away from any contaminations. Transfer the needed volume for assay to a clean vessel and do not return any left-over Substrate to the original bottle.

(6) Buffer, Antibody-enzyme conjugate, OVA-enzyme conjugate, Stop solution
Use the contents of each bottle without any dilution. In the case of storage, keep the bottle closed and store at 2-10°C.

4. Procedure

Keep reagents at room temperature prior to the assay. It is preferable to determine Standard solutions in duplicate.

(1) Dispense the Buffer (150µL) into the Microplate for predispensing samples.
(2) Dispense the sample or Standard solution (15µL) into the Microplate for dispensing samples, and agitate the plate. Then, keep the plate for 10 min at room temperature.
(3) Dispense 110µL of the aliquot solutions prepared at the step (2) into the Antibody coated wells, and then agitate the Antibody coated plate. Incubate the plate covered with an aluminum foil for 60 min at room temperature.
(4) Wash the plate three times with Wash buffer (300µL/well), then tap the plate on a paper towel to remove the Wash buffer. (Do not dry up the plate.)
(5) Add the OVA-enzyme conjugate (100µL) to each well of the plate, and agitate the plate.
(6) Incubate the plate covered with an aluminum foil for 30 min at room temperature.
(7) Wash the plate three times with Wash buffer (300µL/well), then tap the plate on a paper towel to remove the Wash buffer. (Do not dry up the plate.)
(8) Add a 100µL aliquot of Substrate to each well of the plate.
(9) Shield the plate from light and incubate for 30 min at room temperature.
(10) Add a 100µL of Stop solution to each well of the plate at the same interval as the addition of the Substrate, and then agitate the plate. Shield the plate from light.
(11) Measure the absorbance of each well at 450 nm as the main wave length(620 or 630 nm as the reference wave length) within 20 min.

5. Preparation of a standard curve and reading of OVA specific IgE concentration in samples

(1) Graph paper should be used to plot the absorbance on the ordinate and the concentration of each Standard solution on the abscissa. Plot the absorbance obtained by using each Standard solution of the corresponding OVA specific IgE concentration and draw the best-fit smooth curve.
(2) Read the OVA specific IgE concentration corresponding to the absorbance of the sample by using the standard curve.
(3) For quantification of high-concentration samples (more than the concentration of Standard F), dilute the samples properly with the Buffer. Then perform the whole procedure and multiply the obtained value by the dilution factor.

Measurable range

OVA specific IgE: 8.0 – Concentration of Standard F (approximately 800 ng/mL)

Precautions for use or handling

1. General Precautions

(1) Do not use the reagents of this kit in any measure other than those described in this manual. Use for research purposes only.
(2) Do not use reagents beyond the expiration date.
(3) Do not use materials from different kit lot numbers.
(4) Do not freeze the reagents except for the Standard solutions.
(5) Be sure to read the manuals of the instruments used for the assay.
(6) Samples and standard solutions should be treated under the same conditions.
(7) Be careful to avoid contamination between reagents and by microorganisms.

2. Hazards to the user

(1) The reagents in this kit do not contain human-derived materials, and they are thus thought to be negative for HBV, HCV and HIV. However, handle the reagents with care to avoid the possibility of infection by substances in Standard solutions derived from mouse or other animals.
(2) Use this kit under the management of an experienced researcher in this field.
(3) Never use your mouth to pipette the reagents or sample. Always use a pipette with a disposable tip.
(4) Administer emergency treatment such as washing the eyes if any reagent, especially, the Stop solution, in this kit gets in the eyes. In addition, when deemed necessary, receive medical treatment from doctor.
(5) Cooperate to recover kits if unknown infectious agents are found in reagents. In such case, the manufacturer is not responsible for any resulting loss or damage.

Storage method and expiry date

Storage: Store at 2-10°C. Avoid light and freezing.
Expiration date: Printed on box.

Distributor

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Manufacturer

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