| SHIBAYAGI | IgE ELISA Kit For Mouse | Research Reagent |
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**Please, this instruction carefully before use.**

This is a highly sensitive ELISA(Enzyme Linked Immunosorbent Assay) kit for mouse IgE using sandwich method

## [Merit of the Kit]

1. Mouse IgE can be measured rapidly (about 5.5hours).
2. Small volume of sample (serum, plasma*:5μl) is enough
3. Simple assay procedure without any pretreatment of samples.
   *Use heparin to obtain plasma.

## [Contents of the Kit]

| | | | |
|---|---|---|---|
| A: | Antibody-coated plate | 96wells(8x12) | x1 |
| B: | IgE standard solution (100ng/ml) | 600μl | x1 |
| C: | Buffer solution | 60ml | x1 |
| D: | Biotin-conjugated anti-IgE antibody | 10μl | x1 |
| E: | HRP-conjugated avidin | 20μl | x1 |
| F: | Chromogenic substrate reagent(TMB) | 10ml | x1 |
| H: | Reaction stopper (1M $H_2SO_4$) | 10ml | x1 |
| I: | Concentrated washing buffer (10x) | 100ml | x1 |

## [Apparatus Necessary But not Included]

(1) Micropipette (1-1000μl)

(2) Microplate washing apparatsu (microplate washer, shaker, wash bottles, etc.) Microplate reader

## [Preparation of Reagent Solutions]

Use reagent solutions immediately after preparation.
Use all the reagent solutions of the Kit after getting back to room temperature.

(1) Biotin-conjugated anti-IgE antibody:
Prepare by diluting the concentrated solution to 1:1,000 with the buffer solution.

(2) HRP-conjugated avidin
Prepare by diluting the concentrated solutin to 1:2,000 with the buffer solution.

(3) Cromogenic substrate reagent(TMB)
Use the attached solution without dilution.

(4) Concentrated washing buffer
Prepare by diluting concentrated washing buffer to 1:10 with distilled water.

(5) IgE standard solution
Prepare by diluting the original solution as an example shown below.
An example of preparing standard IgE solutions

| Concentration(ng/ml) | 100 | 75 | 50 | 25 | 10 | 1 | 0 |
|---|---|---|---|---|---|---|---|
| *I*gE originail solution(μl) | Orig. | 150 | 100 | 50 | 20 | 5 | 0 |
| Buffer solution(μl) | 0 | 50 | 100 | 150 | 180 | 495 | Buffer |

Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.

## [Assay Procedure]

(1) Wash the plate 3 times with the washing buffer by filling the wells with the buffer and discarding. Thereafter, place the plate upside-down on paper towel for while to remove the excess buffer.

(2) Place 50μl of the standard IgE solutin perpared above to the wells for standard caurve.

(3) Place 45μl of the buffer solution and 5μl of sample to sample wells.

(4) Shake gently using preferably a microplate shaker.

(5) Stand the plate for 2 hr at room temperature(20-25C) for the reaction.

(6) After the reaction, discard the solution, and rinse the plate 3 times with the washing buffer. Thereafter, remove the excess buffer as described above.

(7) Pipette 50μl of the biotin-conjugated antibody solution to each well, and shake gently using preferably a microplate shaker.

(8) Stand the plate for 2 hr at room temperature(20-25C) for the reaction.

(9) After the reaction, discard the solution, and rinse the plate 3 times with the washing buffer. And remove the excess buffer.

(10) Pipette 50μl of HRP-avidin solution to each well, and shake gently on a microplate shaker.

(11) Stand the plate for 1 hr at room temperature(20-25C) for the reaction.

(12) After the reaction, discard the solution, and rinse the plate 3 times with the washing buffer, and remove the excess buffer.

(13) Pipette 50μl of the chromogenic substrate (TMB) reagent to each well, and shake gently on a microplate shaker.

(14) Stand the plate for 20 min at room temperature (20-25C) for the reaction.

(15) Pipette 50μl of the reaction stopper to each well to stop further color development, and shake gently.

(16) Measure absorbance of each well at 450 nm within 30min.

## [Assay Range]

1 to100 ng/ml (with 5 μl sample)

## [Calculation for IgE concentration]

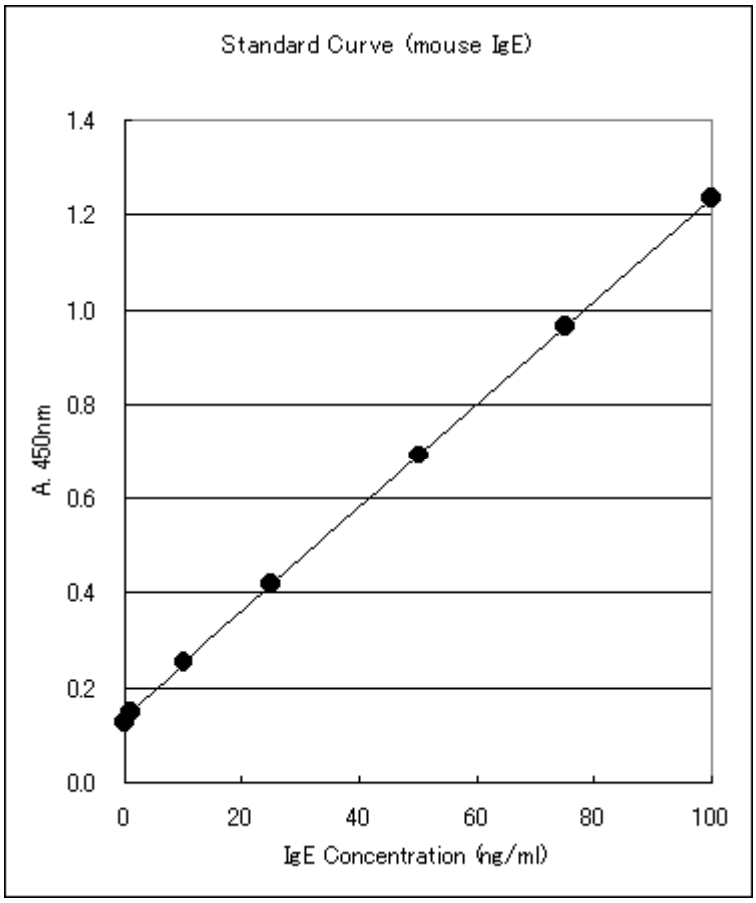
1. Prepare a standard curve by plotting absorbances obtained from standard wells against IgE standard concentrations.
2. Obtain IgE concentrations of the samples from their absorbances.
3. Multiply the IgE concentrations by dilution factors of the samples (in our assay procedure, dilution factor is 10) to obtain the IgE

concentrations of the original samples.

* If a sample absorbance scales out of the standard curve, further dilute the sample and measure again.

* In computer calculation, use 3rd order regression.

## [Standard Curve (an example)]




## [Summary of Assay Procedure]

Antibody-coated plate
Washing
|  + IgE standard solution or Buffer + sample; 50µl
Shaking , Reaction at room temp.(20 - 25C) for 2hr
|
Washing
|  + Biotin-conjugated anti-IgE antibody; 50µl
Shaking , Reaction at room temp.(20 - 25C) for 2hr
|

Washing
|　　+HRP-avidin; 50µl
Shaking , Reaction at room temp.(20 - 25C) for 1hr
Washing
|　　+ Chromogenic substrate solutin; 50µl
Shaking , Reaction at room temp.(20 - 25C) for 20min
|　　+ Reaction stopper; 50µl
Shaking , Measurement of Absorbance(450nm)

## [Reference Assay Value]

Serum level of IgE in intact SPF-NC/Nga male mice of 5 weeks of age, n=24
Mean：85ng/ml,　SD：18ng/ml

## [Statements and Precautions as to Our Kits or Their Components]

(Please start working after reading these notes.)

01. This assay kit or its components should be used only for research works.
02. The reagent solutions of the kit should be used principally immediately after dilution.
Otherwise, keep them in a dark place at 2-8C and use them within 5 days.
03. The reagents were prepared to give accurate results by their combination within the kit. So, do not combine the reagents in the kit of other lot number.
Even the lot number is the same, do not mix the reagents with those that are preserved for some period.
04. Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
05. Do not dry the assay plate to avoid denaturation of the coated antibody or antigen.
06. The reaction time should be counted from the onset of reagent pipetting.
07. Prepare the standard curve in every assay. (For kits with standard solution.)
08. Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
09. Preservation condition for the kit or its components should be strictly kept.
10. Be careful not to allow the reagent solutions of the kit to contact with skin, mucus and eyes (wearing glasses for protection is recommended). Especially treat the stopping solution very carefully because it contains sulfuric acid.
11. HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper should be avoided from contacting with any metal.
12. In treating assay samples of animal origin, be careful for possible

biohazards.

## [Storage Conditions / Term of Validity]

2 - 8C (Shield the kit from the light. Do not freeze.)/ Valid period is indicated on the container.

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