SHIBAYAGI	Mouse Anti-OVA-IgE ELISA KIT	Research Reagent

Please, read this instruction carefully before use.

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of mouse anti-OVA (ovalbumin)-IgE with high sensitivity using Sandwich assay principle.

Measurement of anti-OVA-IgE levels after OVA-inoculation to mouse will be helpful in clarification of the immune process.

[Advantage]

(1) Rapid assay (total reaction time: 1 hour 50min.).
(2) A small sample volume.
(3) An ecologically excellent preservative is used.
(4) Every reagent is provided in liquid form and ready to use.
(5) Excellent precision and reproducibility.
(6) Simple operation without any special pretreatment of samples.

[Components]

	Reagents	Amounts
(A)	OVA-coated plate	96 wells(8x12) / 1 plate
(B)	Standard anti-OVA-IgE solution (1,200U/ml)	100µl / 1 vial
(C)	Buffer solution	60ml/**1** vial
(D)	Biotin-conjugated anti-mouse IgE antibody	200µl/ 1 vial
(E)	Peroxidase-conjugated streptavidin	200µl/ 1 vial
(F)	Chromogenic substrate reagent(TMB)	12ml/ 1 vial
(H)	Reaction stopper (1M H_2SO_4)	12ml/ 1 vial
(I)	Concentrated washing buffer(10x)	100ml/ 1 bottle

[Purpose]

Measurement of anti-ovalbumin IgE antibody in mouse serum or plasma.

[Assay range]

1.88 ~ 120 U/ml

We define 1U of the anti-OVA IgE as 1.3ng of the antibody with an affinity constant (Ka) of 2.0 x $10^8 M^{-1}$.

[Assay sample]

Mouse serum or plasma diluted properly (10 ~ 50x) with buffer (C).

Dilution of the sample will vary according antibody titer.

An example of dilution

	10x	20x	50x
Sample serum or plasma	5 μl	25 μl*	20 μl*
Buffer (C)	45 μl	25 μl	30 μl

* Volume of the solution of one rank higher dilution

[Assay operation]

1. Equipments necessary but not included in the kit.
   (1) Micropipettes able to deliver the sample volume with high precision, and a pipette for repetitive dispensing.
   (2) Microplate washing apparatus (a microplate washer or a flashing bottle with a nozzle).
   (3) A microplate reader (A densitometer for microplate).
   (4) An incubator which can be set at 20 ~ 25 C (preferably)
   (5) A microplate shaker (preferably).
2. Preparation of reagents
   All the reagent solutions should be used after getting back to room temperature (20-25C). We recommend to pre-incubate them for about one hour in an incubator set at 20 ~ 25C.
   (1) Washing buffer: Dilute the concentrated washing buffer (I) to 10X with purified water.
   (2) Biotin-conjugated anti-mouse IgE (D) : Dilute to 100X with the buffer solution(C).
   (3) HRP-conjugated streptavidin (E): Dilute to 100X with the buffer solution(C).
   (4) Other reagents are used as they are.
3. An example of preparing standard solutions
   Dilute the original standard solution (B) with the buffer solution to prepare 120U/ml, then prepare lower standard solutions by serial dilution.

Conc.(U/ml)	120	60	30	15	7.5	3.75	1.88	0
Std. Sol.(μl)	10**	50*	50*	50*	50*	50*	50*	0
Buffer(μl)	90	50	50	50	50	80	50	50

**Original standard solution, *One rank higher standard solution

4. Assay procedure
   (1) Preferably pre-incubate the assay plate in an incubator set at 20~25C for about 1 hour before use.
   (2) Remove the cover sheet of the microplate.
   (3) Rinse the wells (A) by filling the washing buffer and discard 3 times, then strike the plate upside-down onto folded several sheets of paper towel, and remove the excess buffer as completely as possible.
   (4) Pipette 50μl of diluted biotin-conjugated anti-mouse IgE solution into the wells, then shake gently on a plate shaker.
   (5) Pipette 10μl of the standard solution to the wells for preparing a standard curve.
   (6) Pipette 10μl of the diluted sample solution to the wells for samples.
   (7) Shake the plate gently on a plate shaker.
   (8) Incubate for 1 hour at room temperature (20-25C), preferably in the incubator.
   (9) Discard the reaction mixture, and then wash wells and remove the buffer as (3).

(10) Pipette 100μl of HRP-conjugated avidin solution to all wells, and shake as (5).
(11) Incubate the plate for 30 minute at room temperature, preferably in the incubator.
(12) Discard the reaction mixture, and wash the plate and remove the buffer as (3).
(13) Pipette 100μl of chromogenic substrate solution to wells, and shake as (7).
(14) Incubate the plate for 20 minutes at room temperature, preferably in the incubator.
(15) Add 100 μl of the reaction stopper (H) to all wells and shake as (7).
(16) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes.

[Summary of Assay Procedure]

Antibody-coated 96 well plate
↓
Pre-incubate at 20~25C for 1 hour
↓
Washing 3 times
↓
Biotin-conjugated anti-mouse IgE 50μl
↓
Sample or Standard 10μl
↓
Shaking and reaction for 1 hour at 20~25C
↓
Washing 3 times
↓
Peroxidase-conjugated avidin 100μl
↓
Shaking, and reaction for 20 min. at 20~25C
↓
Chromogenic substrate solution 100μl
↓
Shaking, and reaction for 20 min. at 20~25C
↓
Reaction stopper 1M H_2SO_4 100μl
↓
Shaking and measurement of absorbance
at 450nm(sub. 620nm)

[Calculation of anti-OVA-IgE concentration]

(1) Prepare a standard curve using semi-logarithmic or bi-logarithmic section paper by plotting absorbance* (Y-axis) against standard concentration (U/ml) on X-axis.

*Absorbance at 450nm minus absorbance at 620nm.

(2) Using the standard curve, read the anti-OVA-IgE in a sample from its absorbance*, and multiply the assay value by dilution rate if the sample has been diluted. Though the assay range is wide enough, in case the absorbance of some samples are higher than that of the highest standard, please repeat the assay after proper dilution of samples with the buffer solution.

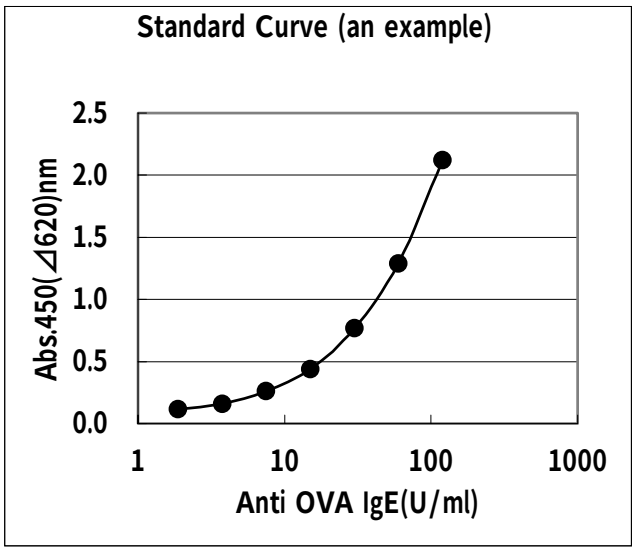
* We recommend the use of 3rd order regression curve or 4 parameter method in computer calculation.

[Important notice in the treatments]

1. Treatment of assay samples
   (1) Use serum or plasma samples obtained by ordinary standard method.
   Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.
   (2) Turbid samples or those containing insoluble materials should be centrifuged before assay and remove those materials.
   (3) Measure the samples as soon as possible after sampling.
   (4) Dilution of the sample should be done using test tubes.
2. Storage of assay samples.
   Assay samples should be kept at 2~8C if assayed within a week. If samples have to be stored for a longer period, snap-freeze samples and store below –35C. Avoid repeated freezing and thawing.
3. Influence of interfering substances
   If presence of interfering substances is suspected, examine by a dilution test using more than 2 points.

[Assay range and assay validation]

1. A model standard curve



2. Specificity

Biotin-conjugated anti-mouse IgE antibody is a specific antibody to mouse IgE..

3. Precision and reproducibility

(1) Within assay variation (2 samples, 5 replicates assay)

Average C.V. is less than 5%.

(2) Reproducibility (3 samples, duplicates assay, 4 days)

Average C.V. is less than 5%.

Precision test

Well	Sample A	Sample B
1	70.7	19.1
2	71.0	18.7
3	77.1	19.6
4	74.3	19.7
5	72.9	18.9
mean.	73.2	19.2
SD	2.6	0.42
CV(%)	3.6	2.2

Unit：U/ml

Reproducibility test

Sample No.	Day 1	Day 2	Day 3	Day 4	Mean	SD	CV%
C	60.0	59.1	58.1	63.6	60.2	2.4	4.0
D	15.0	15.0	14.7	16.0	15.2	0.57	3.7
E	3.75	3.75	3.65	3.48	3.66	0.13	3.4

Unit: U/ml

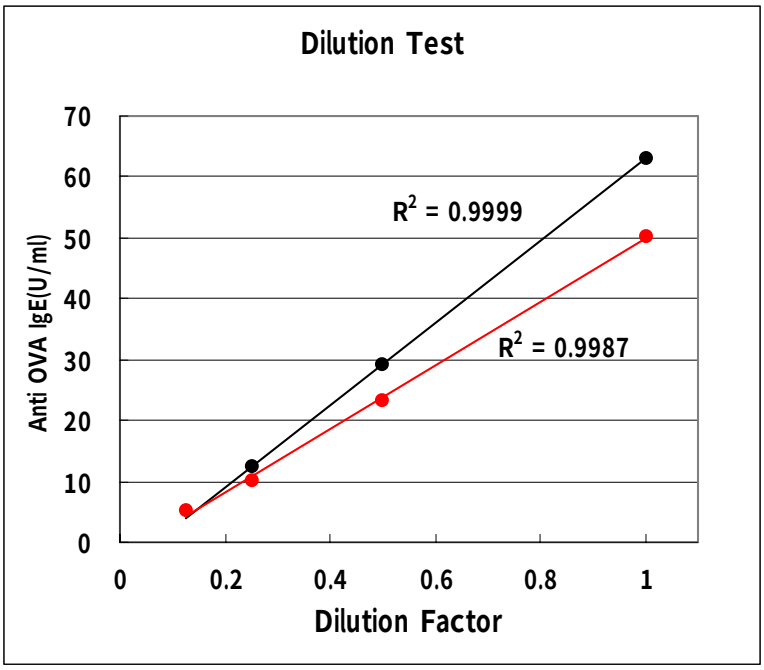
4. Recovery test

Sample No. F			
Added	Found	Recovered	Recovery(%)
0.00	6.93	-	-
5.35	12.2	5.27	98.5
10.7	17.8	10.9	102
17.1	25.1	18.2	106

Sample No. G			
Added	Found	Recovered	Recovery(%)
0.00	40.5	-	-
30.8	70.4	29.5	95.8
35.9	77.1	36.6	102
53.9	94.9	54.4	101

Unit: U/ml, n=3

5. Dilution test



n=2

[Statements and precaution]

(1) The reagents included in this assay kit should be used only for research works.

(2) The reagent solutions of the kit should be used principally immediately after reconstitution. Otherwise, keep them in a dark place with the temperature 2-8C, and use them within 3 days.

(3) 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 have been preserved for some period.

(4) Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.

(5) Do not dry the assay plate to avoid denaturation of the coated antibody.

(6) Measurement of the reaction time should be started from the pipetting of reagent to the first well.

(7) Prepare the standard curve in each assay.

(8) Dilution of the assay sample must be carried out using the buffer solution attached to the kit.

(9) Storage condition for the kit should be strictly followed.

(10) Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Especially be careful for the stopping solution because it is 1M sulfuric acid.

(11) HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper must be avoided from contacting with any metal.

(12) In treating assay samples of animal origin, be careful for possible biohazards.

(13) As the antibody-coated plate is module type of 8wells x 12 rows, each row can be separated by a cutter and used independently.

[Storage condition]

Store the kit at 2~8C. Do not freeze.

[Term of validity]

Six months from production. Expiration date is indicated on the container.

[Unit of package]

96-wells/1 plate

[Product code]

AKRIE-030

Shibayagi Co., Ltd.
1062-1 Ishihara, Shibukawa, Gunma, Japan 377-0007
TEL.+81-279(25)0279, FAX.+81-279(23)0313
URL:http://www.shibayagi.co.jp/
E-mail:syc-info@shibayagi.co.jp