SHIBAYAGI	IgE ELISA Kit For Rat	Research Reagent

Please, read this instruction carefully before use.

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

[Merit of the Kit]

1. Rat IgE can be measured rapidly (about 3.5hours).
2. Small volume of sample (serum, plasma*:5μl) is enough
3. Simple assay procedure without any pretreatment of samples.
4. A wide assay range (1-100ng)
5. Not influenced by hemolysis (hemoglobin concentration less than 40mg/dl).
6. High specificity (Cross reaction with IgG, IgA, IgM less than 0.01%).
   *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	100μl	x1
E:	HRP-conjugated avidin	200μl	x1
F:	Chromogenic substrate reagent(TMB)	12ml	x1
H:	Reaction stopper (1M H_2SO_4)	12ml	x1
I:	Concentrated washing buffer (10x)	100ml	x1

[Apparatus Necessary But not Included]

(1) Micropipette (1-1000μl)

(2) Microplate washing apparatus (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 1:100 with the buffer solution.

(2) HRP-conjugated avidin
Prepare by diluting the concentrated solution to 1:100 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
IgE original 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 wells 3 times with the washing buffer by pipetting 250μl washing buffer and discarding. Thereafter, place the plate upside-down on paper towel for a while to remove the excess buffer.

(2) Place 50μl of the standard IgE solution prepared above to the wells for standard curve.

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

(4) Shake gently using preferably a microplate shaker.

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

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

(7) After the reaction, discard the reaction mixture, and rinse the wells 3 times with each 250μl washing buffer. And remove the excess buffer as stated in (1).

(8) Pipette 100μl of HRP-avidin solution to each well, and shake gently on a

microplate shaker.

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

(10) After the reaction, discard the solution, and rinse the wells 3 times with 250μl washing buffer, and remove the excess buffer.

(11) Pipette 100μl of the chromogenic substrate (TMB) reagent to each well, and shake gently on a microplate shaker.

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

(13) Pipette 100μl of the reaction stopper to each well to stop further color development, and shake gently.

(14) Measure absorbancy of each well at 450 nm within 30min.

[Assay Range]

1 to 100 ng/ml (with 5 μl sample)

[Calculation for IgE concentration]

1. Prepare a standard curve by plotting absorbancies 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.

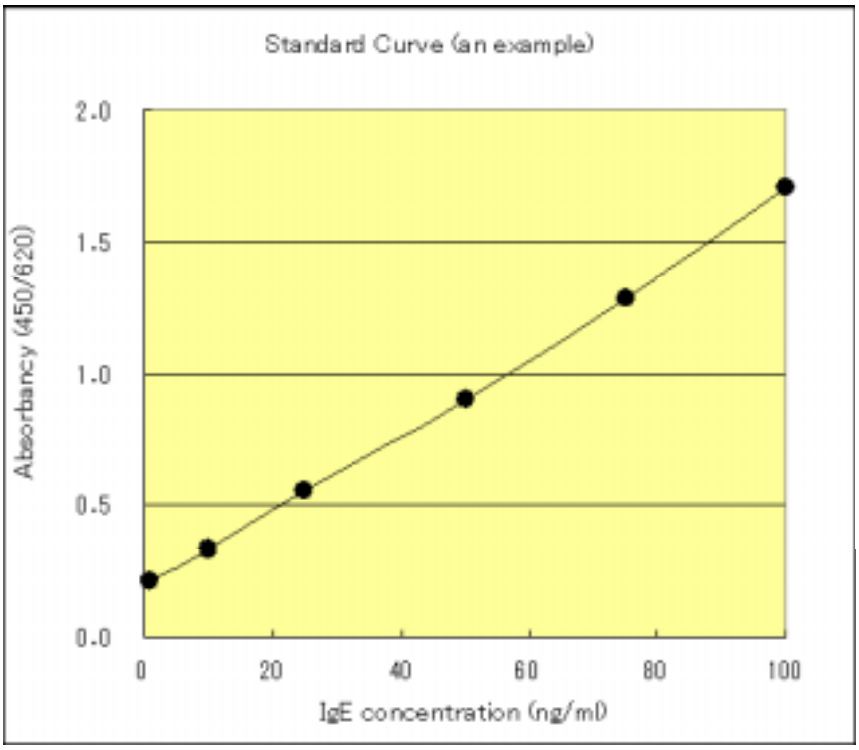
   * If sample absorbances are scale-out of the standard curve, further dilute the sample and measure again.

   * In computer calculation, use 3rd order regression.

[Summary of Assay Procedure]

<u>Antibody-coated plate</u>
Washing
| + IgE standard solution or Buffer + sample; 50μl
Shaking
|
| + Biotin-conjugated anti-IgE antibody; 50μl
Shaking , Reaction at room temp.(20 - 25C) for 2hr
|
Washing
| +HRP-avidin; 100μl

Shaking , Reaction at room temp.(20 - 25C) for 1hr
Washing
| + Chromogenic substrate solution; 100µl
Shaking , Reaction at room temp.(20 - 25C) for 20min
| + Reaction stopper; 100µl
Shaking , Measurement of absorbancy(450nm)



[Storage Conditions / Term of Validity]

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

[Results of validity tests]

[Precision]

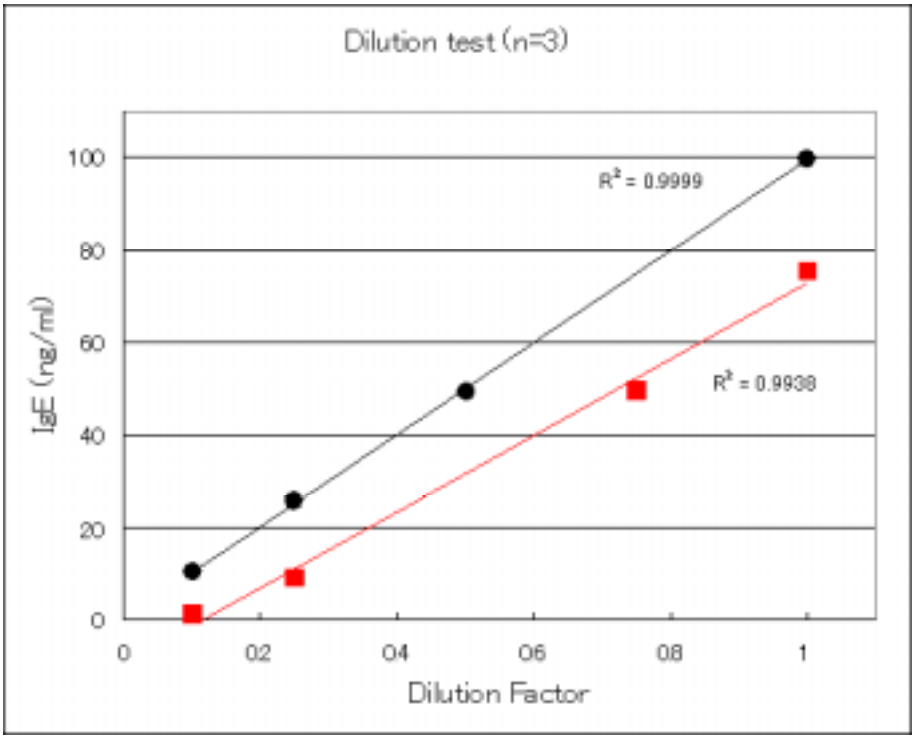
Wells/Samples	A	B	C
1	83.1	37.1	12.8
2	82.1	37.1	12.0
3	80.6	36.5	12.3
4	80.1	35.7	12.7

	5	78.9	35.4	12.5
mean		81.0	36.0	12.5
SD		1.66	0.786	0.370
CV(%)		1.9	1.9	1.8
			(ng/ml)	n=5

【Reproducibility】

Sample ID	A-2	B-20	N-19
Day 1	375	135	74.7
Day 2	365	134	71.2
Day 3	400	144	77.0
Day 4	371	138	74.8
mean	378	138	74.4
SD	15.4	4.73	2.40
CV(%)	4.1	3.4	3.2
		(ng/ml)	n=3

【Dilution test】



[Assay data]

IgE in male SD rats of 5weeks old, n=7, serum sample (Shibayagi's data)

Mean: 45.5 ng/ml, (Standard deviation: 6.2ng/ml)

The value may change by keeping conditions.

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