SHIBAYAGI	Mouse DNP-IgE ELISA Kit	Research Reagent

Please, read this instruction carefully before use.

If you immunize mice with DNP (dinitrophenol)-carrier as immunogen under various treatments, and examine anti-DNP-IgE antibody, anti-DNP-IgG antibody, and total IgE, you can estimate the effects and mode of action of those treatments on mouse immune system.

This is a kit for measurement of mouse anti-DNP-IgE type antibody by ELISA (Enzyme-linked immunosorbent assay).

[Merit of the kit]

This assay kit can measure anti-DNP-IgE,

(1) Quickly (within 3 hours),
(2) With a small volume of sample (10 μl),
(3) Promptly and easily with all reagents provided as solution,
(4) With high precision.

[Kit components]

Components	Amount
(A) DNP-coated plate	96wells(8x12)/1 plate
(B) Standard solution (50000ng/ml)	100 ml/1 bottle
(C) Buffer	60ml/1 bottle
(D) Biotin labeled anti-IgE antibody	100μl/1 bottle
(E) Avidin-HRP conjugates	200μl/1 bottle
(F) Chromogenic substrate (TMB)	12ml/1 bottle
(H) Reaction stopper (1M H2SO4)	12ml/1 bottle
(I) Concentrated washing solution (10X)	100ml/1 bottle

[Purpose of the kit]

Measurement of anti-DNP-IgE type antibody in mouse serum (or plasma)

[Operation of the kit]

1. Equipments necessary but not included.
   (1) Micropipette (1-1000μl, etc.)
   (2) Washing apparatus for microplate (a microplate washer, or a jet bottle, etc.)
   (3) Microplate reader

2. Preparation of reagents
   (1) Concentrated washing solution(I): Dilute 1 volume of the original concentrated solution with 9 volumes of purified water.
   (2) Biotin-labeled anti-IgE (D): Dilute 1volume of the original solution with 99 volumes of buffer.
   (3) Avidin-HRP conjugates (E):Dilute 1volume of the original solution with 99 volumes of buffer.
   (4) Other reagent solutions, except standard solution (B), can be used as

they are, without dilution.

Caution ! :Use all the reagents after getting back to room temperature.

3. Dilution of assay samples and standard solution(B)
   (1) Assay samples: Dilute assay samples, if necessary, with buffer.
   (2) Preparation of standard solutions: Dilute original standard solution with buffer as shown in an example.

Standard conc.(ng/ml)	5000	1000	500	100	50	10	0
Standard solution (μl) (See note!)	20*	50**	100**	50**	100**	50**	0
Buffer	180	200	100	200	100	200	150

Note: * Original solution, **Standard solution of one step higher concentration.

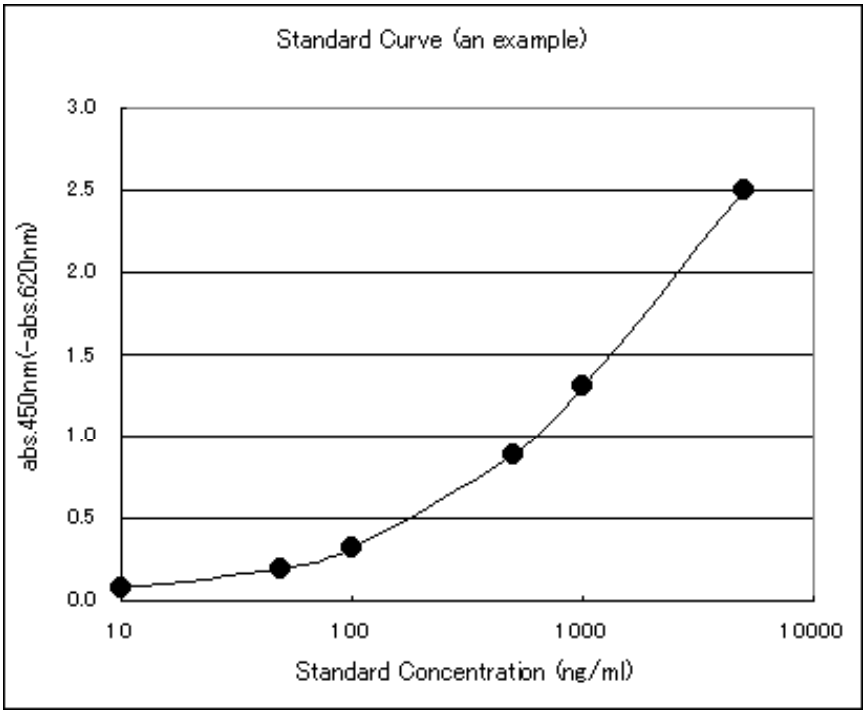
4. Assay procedure
   (1) Remove the sheet covering the microplate, then shake off the protective liquid in the well.
   (2) Using pipette (250μl) or jet bottle, fill the wells with washing buffer prepared as above. Shake off the buffer, then again fill the wells with washing solution and shake off. Repeat this once more (total 3 times washing). Then hold the microplate upside-down, and tap dowon the microplate several times on paper towel to remove the remaining washing solution.
   (3) Pipette 50μl of standard solutions or samples to wells.
   (4) Using a microplate shaker, shake the plate gently. If microplate shakers are not available, place the plate on a flat surface of the desk, hold both ends of the plate by a hand, and move the plate like drawing circles (about twice a second) for about ten seconds.
   (5) Stand the plate for 1 hr at room temperature (20-25C).
   (6) Discard the reaction mixture, and wash the plate as (2).
   (7) Pipette 50μl of Biotin labeled anti-IgE solution prepared above to each well.
   (8) Shake plate as (4).
   (9) Stand the plate for 1hr at room temperature (20-25C).
   (10) After the reaction, discard the reaction mixture, and wash the plate as (2).
   (11) Pipette 50μl of Avidin-HRP solution prepared above to each well.
   (12) Shake the plate as (4).
   (13) Stand the plate for 30mins at room temperature (20-25C).
   (14) After the reaction, discard the reaction mixture, and wash the plate 3 times as (2).
   (15) Pipette 50μl of chromogenic substrate solution to each well.
   (16) Shake the plate as (4).
   (17) Stand the plate for 20mins at room (20-25C).
   (18) Pipette 50μl of the stopper solution to each well.
   (19) Shake the plate as (4).
   (20) Measure the absorption at 450nm (with sub-wave length 620nm) using a microplate photometer.

[Summary of the procedure]

DNP-coated well plate
|
Washing, 3 times
|←Standards or samples 50μl

Shaking
|
Reaction, 1 hr at room temperature
|
Washing, 3 times
|←Biotin labeled anti-IgE 50µl
Shaking
|
Reaction, 1 hr at room temperature
|
Washing, 3 times
|←Avidin-HRP 50µl
Shaking
|
Reaction, 30mins at room temperature
|
Washing, 3 times
|←Chromogenic substrate 50µl
Shaking
|
Reaction, 20mins at room temperature
|← Reaction stopper 50µl
Skaking
|
Measurement of absorption at 450nm
(sub-wavelength 620nm)

[An example of standard curve]



[Calculation of assay value]

Prepare a standard curve using semi-logarithmic section paper with Y axis as 450nm absorbance (or 450nm absorbance-620nm absorbance), and X axis as logarithmic concentration of standard DNP-IgE. An example is shown above.

(1) Using the standard curve, read the assay values of samples corresponding to their absorbance.

(2) Serum samples should be assayed after proper dilution for their final absorbance to be within the assay range.
If you dilute the sample before assay, the original DNP-IgE level in the sample can be obtained by (assay value reading from the standard curve x dilution factor).

[Important notes for operation]

1. Treatment of samples
   (1) Use serum or plasma* samples prepared with standard procedure.
   *an example of anticoagulant: 10-100μg heparin/ml blood.
   Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.
   (2) Insoluble matters in samples should be removed by centrifugation or filtration.
   (3) Samples should be assayed as soon as possible after preparation.

2. Storage of assay sample
   We recommend immediate assay after sampling. If not assayed immediately, the samples should be kept in a refrigerator at 2-8C within a week, or frozen below -20C for a longer period.. Avoid repeated freezing and thawing.

3. Checking influence of interfering substances
   If the presence of any interfering substance is suspected, assay the same sample after several dilution, and confirm the linearity of their assay values. (See dilution test below).

[Assay range and assay validation]

1. Assay range
   Absorbance corresponding standard concentration 10 ー5,000ng/ml is 0.05 ー 2.5.

2. Specificity
   Biotin-labeled IgE antibody is a specific antibody to mouse IgE.

3. Assay precision (Intra-assay variation, 3 samples, n=8)
   Average CV is less than 5%.

	Sample ID		
Well	A	B	C
1	778	171	41.0
2	749	171	42.3
3	762	174	43.2
4	765	172	45.1
5	778	171	43.5
6	792	175	45.1
7	753	175	43.5
8	803	164	47.8
Mean	772	172	43.9
SD	19	3.4	2.1
CV(%)	2.4	2.0	4.8

unit: ng/ml

4. Reproducibility (Inter-assay variation, 4 samples, triplicated assay)
(Average CV is less than 5%)

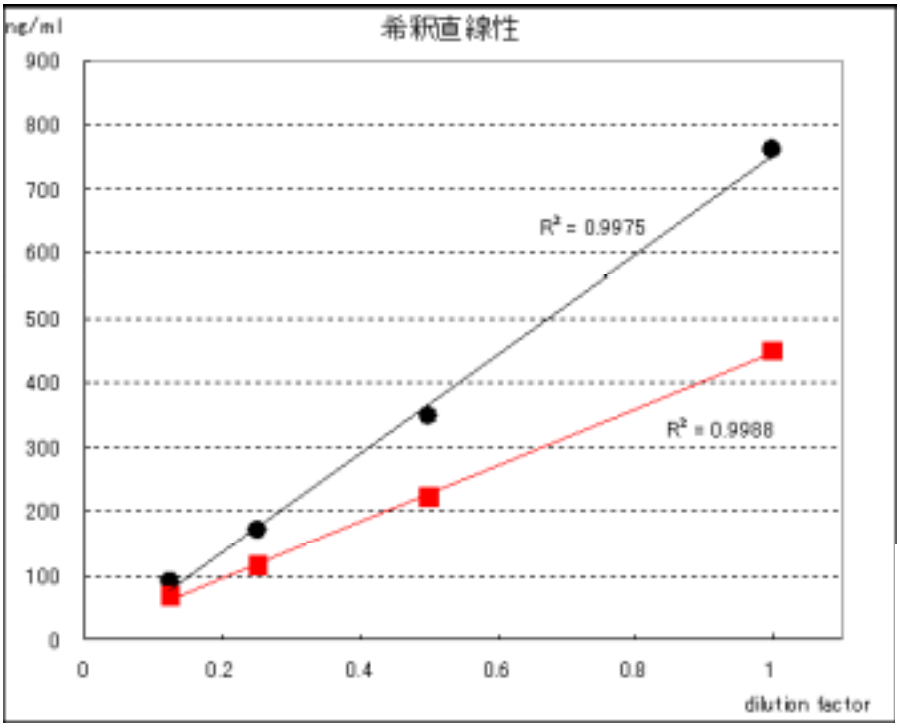
	Sample ID			
Assays	H	I	J	K
Day 1	991	508	95.8	50.9
Day 2	1035	507	94	53.3
Day 3	1000	501	97.4	53.1
Day 4	1000	501	97.8	52.5
Mean	1006	504	96.3	52.5
SD	19.7	3.73	1.73	1.06
CV (%)	0.17	0.74	1.8	2

unit: ng/ml

5. Recovery test (n=4)

Added (ng/ml)	Found (ng/ml)	Recovered (ng/ml)	Rates (%)
0	40.2	-	-
52.1	91.0	50.8	97.5
213	260	220	103
592	632	592	100
1003	1073	1033	103

6. Dilution test (2 samples, n=2)



[Important notes about kit]

1. General caution
   (1) Do not expose the kit to the direct sunlight
   (2) Do not freeze the kit.
   (3) Use every component of the kit as soon as possible after opening of the bottle. If storage is necessary, close the bottles firmly, and keep them under the storage condition as is indicated. Dilution of the reagent should be done immediately before use.
   (4)Do not use the reagents of this kit with those of other lots. Do not use the reagents for other purposes than measuring DNP-IgE..
   (5) Do not use wet apparatus
   (6) Do not use the kit after expiration date.
   (7) Use all the reagents and samples after getting back to room temperature.
   (8) After assays, the rest of the assay samples, the microplate, and pipette tips and other materials should be discarded after sterilization by immersing them for more than 1 hour in either 1% formalin, 2% glutalaldehyde, or sodium hypochlorite (more than 0.1%).

2. About assay values of the kit
   (1) The assay values of the kit is expressed by weight of a purified monoclonal DNP-IgE which was calibrated by using Shibayagi's IgE assay kit.

[Storage condition/Term of Validity]

Please store this kit at 2-8C avoiding freezing.
This kit can be used until 6 months after preparation.
The expiration date is indicated on the label of the container.

[Package unit]

96wells/plate (Product code: AKRIE-020)

Please, check "Statements and Precautions as to Our Kits or Their Components" in a separate page for further information.

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