SHIBAYAGI	Mouse Anti-OVA-IgG$_1$ 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)-IgG$_1$ with high sensitivity using Sandwich assay principle.

Measurement of anti-OVA-IgG$_1$ 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-IgG$_1$ solution (1,200mU/ml)	100μl / 1 vial
(C)	Buffer solution	60ml/　vial
(D)	Biotin-conjugated anti-mouse IgG$_1$ antibody	200μl/ 1 vial
(E)	Peroxidase-conjugated streptavidin	200μl/ 1 vial
(F)	Chromogenic substrate reagent(TMB)	12ml/ 1 vial
(H)	Reaction stopper (1M H$_2$SO$_4$)	12ml/ 1 vial
(I)	Concentrated washing buffer(10x)	100ml/ 1 bottle

[Purpose]
Measurement of anti-ovalbumin IgG$_1$ antibody in mouse serum or plasma.

[Assay range]
1.88 ~ 120 U/ml

We define 1U of the anti-OVA IgG$_1$ as 160ng of the antibody with an affinity constant (Ka) of 6.9 x 10^{7}M^{-1}.

[Assay sample]
Mouse serum or plasma diluted properly (100 ~ 10,000x) with buffer (C).

Dilution of the sample will vary according antibody titer.

An example of dilution

	10x	100x	1,000x	10,000x
Sample serum or plasma	5 μl	20 μl*	20 μl*	20 μl*
Buffer (C)	45 μl	180 μl	180 μl	180 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 IgG$_1$ (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.(mU/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 IgG$_1$ 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.

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(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 IgG$_1$ 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$_2$SO$_4$ 100μl

Shaking and measurement of absorbance
at 450nm(sub. 620nm)

[Calculation of anti-OVA-IgG$_1$ 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-IgG$_1$ 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

Standard Curve (an example)
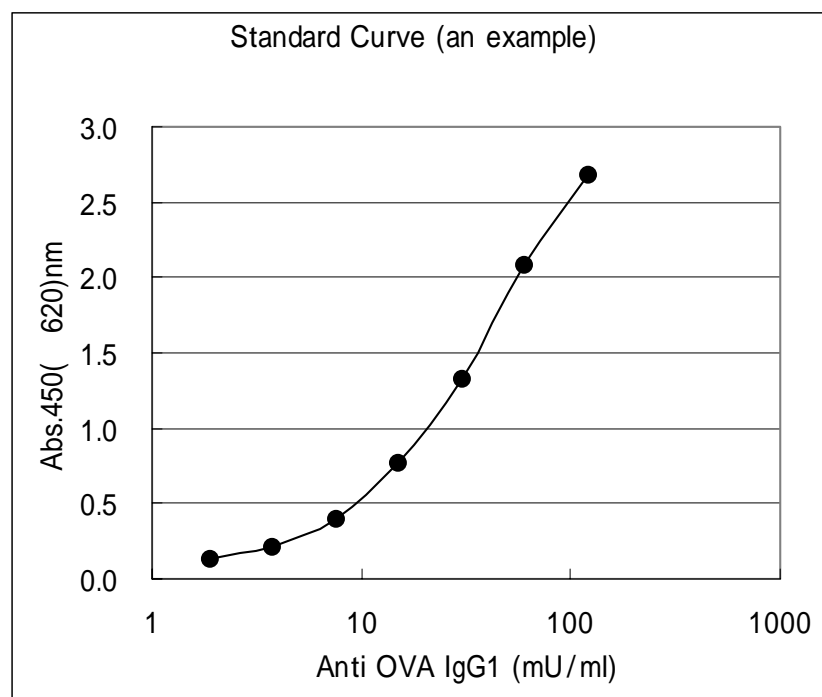
2. Specificity

Biotin-conjugated anti-mouse IgG$_1$ antibody is a specific antibody to mouse IgG$_1$.

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	105	18.2
2	100	16.9
3	96.2	16.7
4	100	17.4
5	106	17.4
mean.	101	17.3
SD	4.0	0.59
CV(%)	4.0	3.4

Unit   mU/ml

Reproducibility test

Sample	Day 1	Day 2	Day 3	Day 4	Mean	SD	CV(%)
C	60.1	58.5	59.9	61.4	60.0	1.2	2.0
D	15.0	14.7	15.3	15.7	15.2	0.44	2.9
E	3.75	3.70	3.83	3.82	3.77	0.06	1.6

Unit: mU/ml

4. Recovery test

Sample No. F			
Added	Found	Recovered	Recovery(%)
0.00	8.86	-	-
7.29	15.8	6.94	95.2
11.0	19.5	10.6	96.4
14.6	23.0	14.1	96.6

Sample No. G			
Added	Found	Recovered	Recovery(%)
0.00	51.5	-	-
26.9	76.8	25.3	97.1
31.4	80.6	29.1	92.5
47.1	100	48.5	103

Unit   mU/ml   n=3

5. Dilution test

Dilution Test

(Y-axis: Anti OVA IgG (mU/ml), values 0, 20, 40, 60, 80, 100, 120)
(X-axis: Dilution Factor, values 0, 0.2, 0.4, 0.6, 0.8, 1)

$R^2 = 0.9994$

$R^2 = 0.9998$
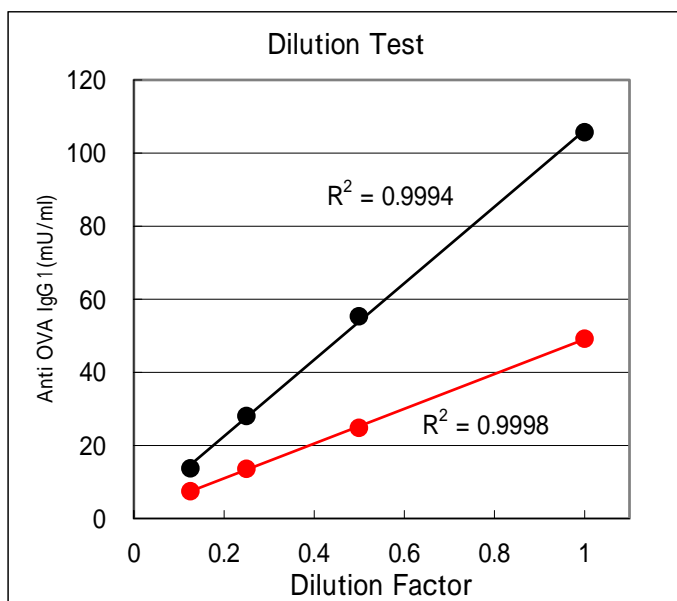
n=2

[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-040

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