SHIBAYAGI	Mouse Resistin ELISA Kit	Research Reagent

Please, read this instruction carefully before use.

This is an ELISA (Enzyme Linked Immunosorbent Assay) kit for measurement of mouse resistin with high sensitivity using Sandwich assay principle.

[Advantage]

（1） Rapid assay (total reaction time: 2 hours 50min.).
（2） A small sample volume (10μl).
（3） An ecologically excellent preservative is used.
（4） Every reagent is provided in liquid form and ready to use.
（5） Excellent precision and reproducibility.

[Components]

	Reagents	Amounts
(A)	Anti-resitin-coated plate	96 wells(8x12) / 1 plate
(B)	Standard resisitin solution (500ng/ml)	200μl / 1 vial
(C)	Buffer solution	60ml/1 vial
(D)	Biotin-conjugated anti-resistin	100μl/ 1 vial
(E)	Peroxidase-conjugated streptavidin	100μl/ 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

[Assay samples]

Mouse serum or plasma. 10μl/well (in standard assay)
Or diluted 5x with assay buffer before assay (1vol. sample+4vol buffer). 50μl/wel

[Assay range]

0.5 ~ 50ng/ml

[Assay operation]

1. Equipments necessary but not included in the kit.

(1) Micropipette (we recommend to use a micropipette prepared for 10μl or less for sampling.)
(2) Microplate washing apparatus (a microplate washer or a nozzled flashing bottle)
(3) A microplate reader (A densitometer for microplate).

2. Preparation of reagents

(1) Washing buffer: Dilute the concentrated washing buffer (I) to 10X with purified water.

(2) Biotin-conjugated anti-resistin (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.

(5) All the reagent solutions should be used after getting back to room temperature (20-25C).

3. An example of preparing standard solutions

Dilute the original standard solution (B) with the buffer solution to prepare 50ng/ml, then prepare lower standard solutions by a dilution program shown below.

Concentration(ng/ml)	50	25	10	5	2.5	1	0.5	0
Std. Sol. (μl)	50 (orig.sol)	250*	200*	250*	250*	200*	250*	0
Buffer (μl)	450	250	300	250	250	300	250	250

*One rank higher standard solution

4. Assay procedure

Remove the cover sheet of the microplate after getting back to room temperature.

(1) Rinse the anti-resistin coated 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.

(2) Pipette 40μl of buffer solution into the wells for samples, then add 10μl of sample. Alternatively, if samples are already diluted to 5X, pipette 50μl of the diluted sample to each well, skipping the addition of buffer solution.

(3) Pipette 50μl of the standard solution to the wells for preparing a standard curve.

(4) Shake the plate gently on a plate shaker for 10-15 seconds.

(5) Incubate for 1 hour at room temperature (20-25C).

(6) Discard the reaction mixture, and then wash wells as described in (1).

(7) Pipette 50μl of biotin-conjugated anti-resistin solution to all wells. Then shake gently on a plate shaker as (4).

(8) Incubate the plate for 1 hour at room temperature.

(9) Discard the reaction mixture, and then wash the plate as (1).

(10) Pipette 50μl of HRP-conjugated avidin solution to all wells, and shake as (4).

(11) Incubate for 30 minute at room temperature.

(12) Discard the reaction mixture, and wash the plate as (1).

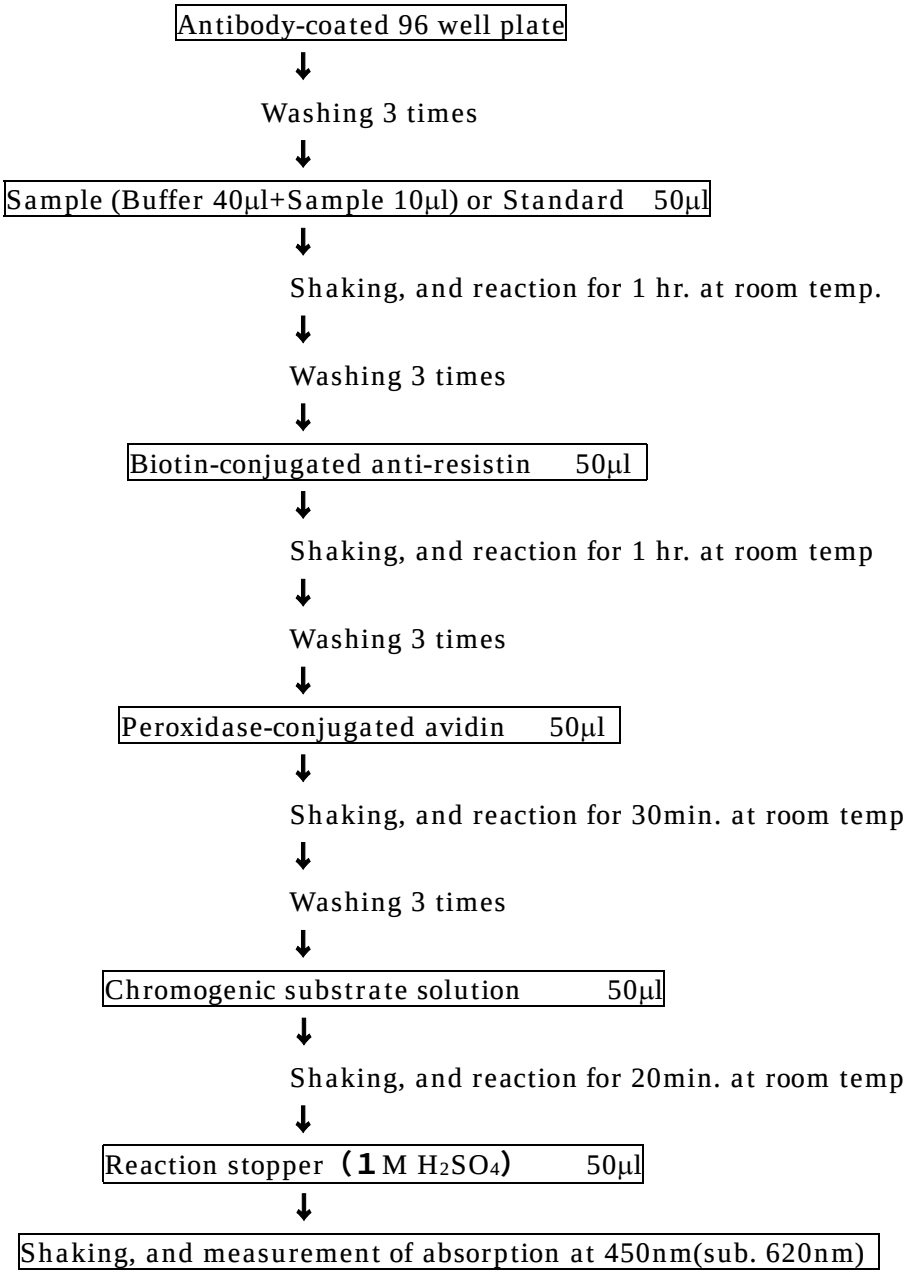
(13) Pipette 50μl of chromogenic substrate solution to wells, and shake as (4).

(14) Let the plate stand for 20 minutes at room temperature.

(15) Add 50 μl of the reaction stopper (H) to all wells and shake.

(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]



Room temp.: 20~25C

[Calculation of resistin concentration]

(1) Prepare a standard curve using section paper by plotting absorbance (Y-axis) against resistin concentration (ng/ml) on X-axis.

(2) Using the standard curve, read the resistin concentration of a sample from its absorbance. Multiply this value by 5 because the sample is diluted 5X in the standard procedure.

(Semi-logarithmic section paper and logarithmic section paper are also useful for lower concentration area.)

* Though the assay range is very wide, 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.

2. Storage of assay samples.

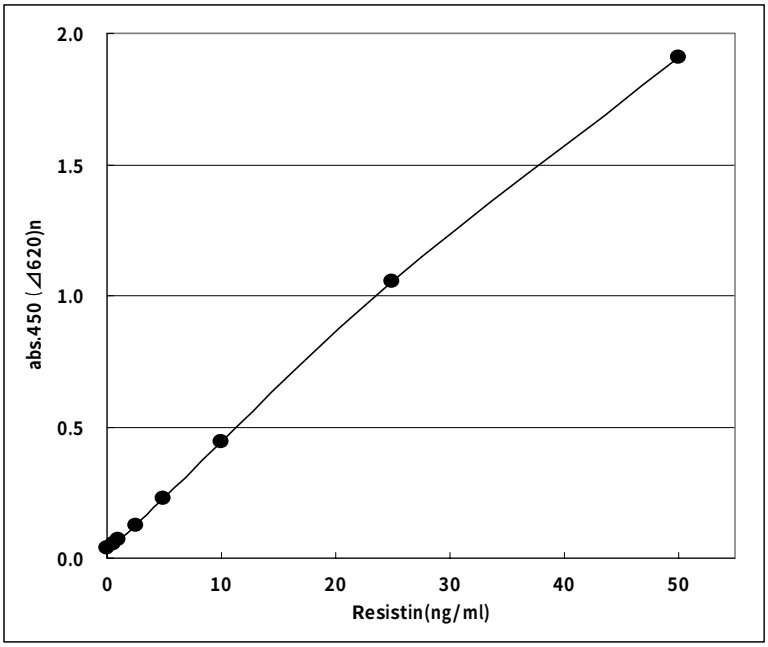
If assay samples have to be stored for a long period, freeze samples and store below –35C. Avoid repeated freezing and thawing.

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

In this assay kit we use the specific antibodies to resistin obtained by affinity chromatography..

3. Precision and reproducibility

(1) Within assay variation (2 samples, 4 fold assay)
Average C.V. is less than 5%.

(2) Reproducibility (3 samples, triplicates assay, 4 days)
Average C.V. is less than 5%.

[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 no 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]

6 month from production. Expiration date is indicated on the container.

[Unit of package]

96-wells/1 plate (Product code: AKRRS-011)

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