KLH (TDAR) Rat-IgM ELISA KIT

Research Reagent

For in-vitro laboratory use only

Please, read this instruction carefully before assay.

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of IgM-type anti-KLH (Keyhole limpet hemocyanin) antibody after inoculation of KLH to rat with high sensitivity using Sandwich assay principle. This is helpful in checking TDAR T-cell dependent antibody reaction) in combination with KLH (TDAR) Rat-IgG ELISA KIT.

Advantage

(1) Rapid assay (total reaction time:2hours 20min.).
(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.

Components

	Reagents	Amounts
(A)	KLH-coated plate	96 wells(8x12) / 1 plate
(B)	Standard anti-KLH rat IgM solution (1000ng/ml)	200µl / 1 vial
(C)	Buffer solution	100ml/ 1 bottle
(D)	HRP-conjugated anti-rat IgM	100µl/ 1 vial
(F)	Chromogenic substrate reagent (TMB)	12ml/ 1 bottle
(H)	Reaction stopper (1M H_2SO_4)	12ml/ 1 bottle
(I)	Concentrated washing buffer (10x)	100ml/ 1 bottle

Assay sample

Rat serum or plasma diluted with buffer (C) to be in the assay range.
*Dilution shall be changed according to the experimental situation.
Example of sample dilution series

Dilution rate	Preliminary 20X	200X	2000X	20000X
Serum/Plasma	10µl	20µl*	20µl*	20µl*
Buffer (C)	190µl	180µl	180µl	180µl

*: One rank higher sample solution

Assay operation

1. Equipments necessary but not included in the kit.

(1) Micropipette (a micropipette able to deliver sample volume with high precision.), and a pipette for repetitive dispensing.
(2) Microplate washing apparatus (a microplate washer or a flashing bottle with nozzle).
(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) HRP-conjugated anti-rat IgM (D):Dilute to 100X with the Buffer solution(C).
(3) Other reagents are used as they are.
(4) Use all the reagent solutions after bringing them up to room temperature (20-25C).

3. An example of preparing standard solutions

Dilute the original standard solution (B) with the buffer solution to prepare 200 ng/ml, and then prepare lower standard solutions by a dilution program shown below.
(You can use other mode of dilution for a set of standard solutions.)

Std.Conc.(ng/ml)	200	100	50	25	12.5	6.25	3.13	0

Standard. sol.(μl)	100**	200*	200*	200*	200*	200*	200*	0
Buffer sol.(μl)	400	200	200	200	200	200	200	200

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

4. Assay procedure

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

(1) Rinse the KLH coated wells (A) by filling the wells with washing buffer and discard 3 times. Remove the residual buffer in the wells by striking the plate upside-down onto several sheets of folded paper towel.
(2) Pipette 50μl of the standard solution and diluted sample solution to the assigned wells for preparing standard curve and those for samples, respectively.
(3) Shake the plate gently on a plate shaker.
(4) Incubate for 1 hour at room temperature (20-25C).
(5) Discard the reaction mixture, and then wash the plate 3 times, and remove the residual washing buffer in the wells as in (1).
(6) Pipette 50μl of HRP-conjugated anti-rat IgM antibody solution (D) to all wells. Then shake the plate gently on a plate shaker as (3).
(7) Incubate the plate for 1 hour at room temperature.
(8) Discard the reaction mixture, and then wash the plate 3 times, and remove the residual washing buffer in the wells as in (1).
(9) Pipette 50μl of Chromogenic substrate reagent to wells, and shake as (3).
(10) Let the plate stand for 20 minutes at room temperature.
(11) Add 50 μl of the Reaction stopper (H) to all wells and shake as (3).
(12) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes. Use the value obtained by subtracting Abs.620 from Abs.450.

Summary of Assay Procedure

KLH-coated plate (wells)
↓
Washing 3 times
↓
Sample or Diluted Standard 50μl
↓
Shaking on a plate shaker for 10-15 seconds.
↓
Reaction for 1 hour at room temp.
↓
Washing 3 times
↓
HRP-conjugated anti-rat IgM antibody 50μl
↓
Shaking on a plate shaker for 10-15 seconds.
↓
Reaction for 1 hr. at room temp.
↓
Washing 3 times
↓
Chromogenic substrate reagent 50μl
↓
Shaking on a plate shaker for 10-15 seconds.
↓
Reaction for 20 mins. at room temp.
↓
Reaction stopper 1M H_2SO_4 50μl
↓
Shaking on a plate shaker for 10-15 seconds.
↓
Measurement of absorbance at 450nm(sub. 620nm)

Room temp.: 20~25C

Calculation of anti-KLH rat IgM concentration

(1) Prepare a standard curve by plotting absorbance* (Y-axis) against standard concentration (ng/ml) on X-axis. For the manual reading from the standard curve, we recommend the use of bi-logarithmic section paper.
*Absorbance at 450nm minus absorbance at 620nm.
(2) Read anti-KLH rat IgM concentration of a sample from its absorbance*, and multiply the assay value by dilution rate. Though the assay range is wide enough, in case the absorbances 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 parameters 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.
(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 (absorbance may change depending on assay condition)

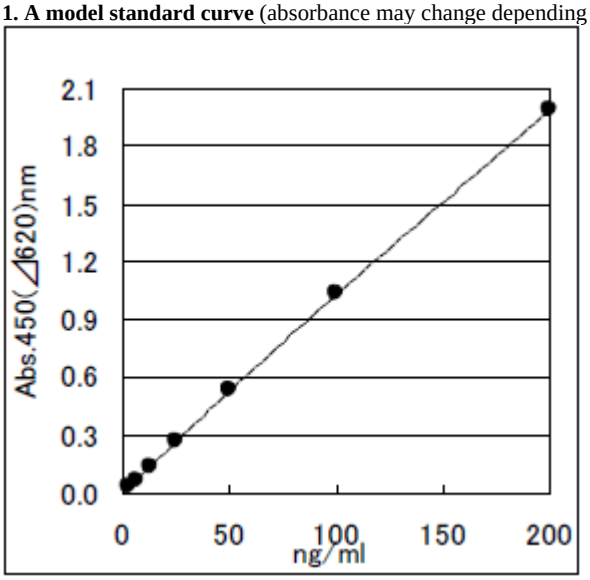

Plate reader: SUNRISE RAINBOW (TECAN), 3rd order regression
Lower concentration area can be expanded if higher concentration area is eliminated..

2. Specificity

As HRP-conjugated anti-rat IgM antibody is used, cross-reaction to IgG is less than assay sensitivity.

3. Precision and reproducibility

(1) Precision (intra- assay variation , 2 samples, 5 replicates assay) Average CV is less than 5%.
(2) Reproducibility (inter-assay variation, 4 replicates assay, 3 samples, 4 days) Average CV is less than 5%.

Notice

1. General caution on kits

(1) Place this kit keeping away from direct sunshine or heat.
(2) Do not freeze the kit.
(3) After opening the containers of reagents, use them as soon as possible. When storing, keep the tightly capped bottles under the temperature of 2~8C in a dark place.
(4) The reagents of the kit must not be used for other purpose than ELISA.
(5) Use fully dried lab-wares (pipette tips, glasswares) in assay.
(6) Temperature should be strictly kept in the range of 20~25C during assay process.

2. Precaution about kit and assay results

(1) Do not use the kit after its expiration date.
(2) The reagents were prepared specifically for each lot in order to give accurate results. So, do not combine the reagents in the kit of other lot number. Even if the lot number is the same, do not mix the reagents with those that have been preserved for some period. Before starting assay, every reagent should be brought up to the room temperature (20~25C). Incomplete temperature condition may influence the assay results and cause incomplete performance of the kit.
(3) Judgment of the assay results should be carefully made from the overall situation including condition of the animals and results of other examinations.

3. Precaution in treatments

(1) In treating assay samples of animal origin, be careful for possible biohazards.
(2) After assay, samples and waists should be dipped in 1% formalin, 2% glutaraldehyde, or more than 0.1% sodium hypochlorite solution for more than 1 hour before discarding them. Alternatively, treat them in autoclave for sterilization.
(3) Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Take special care when handling the stopping solution because it is 1M sulfuric acid.
(4) If such hazard happens, wash the place with enough volume of water, and if necessary, consult a doctor as soon as possible.

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

AKRKM-010

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