Mouse Anti-ssDNA ELISA KIT

Research Reagent

For in-vitro laboratory use only

Please, read this instruction carefully before assay.

Reagents

A: Mouse ssDNA coated plate	96well(8x12)	x1
B: Standard Mouse-antibody solution (10U/ml)*	100µl	x1
C: Buffer solution	60ml	x1
D: HRP-conjugated anti-Mouse-IgG	20µl	x1
E: Chromoqenic substrate reagent (TMB)	12ml	x1
F: Reaction stopper (1M H_2SO_4)	12ml	x1
G: Concentrated washing buffer (10x)	100ml	x1

* Concentration may be different according to Lot.

Apparatus

(1) Micropipette (1-1000µl)
(2) Microplate washing apparatus (microplate washer, shaker, wash bottles,etc.)
(3) Microplate reader

Preparation of Reagent Solutions

(1) Washing buffer: Prepare by diluting concentrated washing buffer to 1:10 with distilled water.
(2) HRP-conjugated antibody solution:Prepare by diluting the concentrated solution to 1:2000 with the assay buffer.
(3) Other reagents can be used without dilution.
(4) Use all the reagent solutions of the Kit after returning to room temperature.

Dilution of Assay Samples and Preparation of the Standard Antibody Solution

(We show an example)
(1) Sample:Mouse serum or plasma(Heparin is not suitable for this assay system.)
Dilute to 1:51,1:101 or 1:201 with the assay buffer.
(2) Standard antibody solutions (The first dilution of the original standard solution may be changed according to the concentration indicated)
Prepare standard antibody solutions by serial dilution of the antibody solution with the assay buffer as follows.

Potency(mU/ml)	1000	500	250	125	62.5	31.3	15.6	0
Standard solution(µl)	50	250	250	250	250	250	250	0
Assay buffer(µl)	450	250	250	250	250	250	250	250

Assay Procedure

(1) Wash the plate 3 times with the washing buffer by filling the wells with the buffer and discarding. Thereafter, place the plate upside -down on the paper towel for a while to remove the excess buffer.
(2) Place 100µl of the standard antibody solution or diluted sample to each well.
(3) Shake gently using preferably a microplate shaker.
(4) Stand the plate for 1 hours at room temperature (20-25C) for the reaction.
(5) After the reaction, discard the solution, and rinse the plate 3 times with the washing buffer. Then remove excess buffer as described above.
(6) Pipette 100µl of the HRP-conjugated antibody solution to each well, and shake gently using preferably a microplate shaker.
(7) Stand the plate for 1 hours at room temperature (20-25C) for the reaction.
(8) After the reaction, discard the solution, and rinse the plate 3 times with the washing buffer. Then remove excess buffer as described above.
(9) Pipette 100µl of the chromogenic substrate (TMB) reagent to each well, and shake gently on a microplate shaker.
(10) Stand the plate for 20 minutes at room temperature (20-25C) for the reaction.
(11) Pipette 100µl of the reaction stopper to each well to stop further color development.

(12) Measure absorbance of each well at 450 nm (Sub-wavelength, 620nm).

Storage condition

Store at 2 to 8C

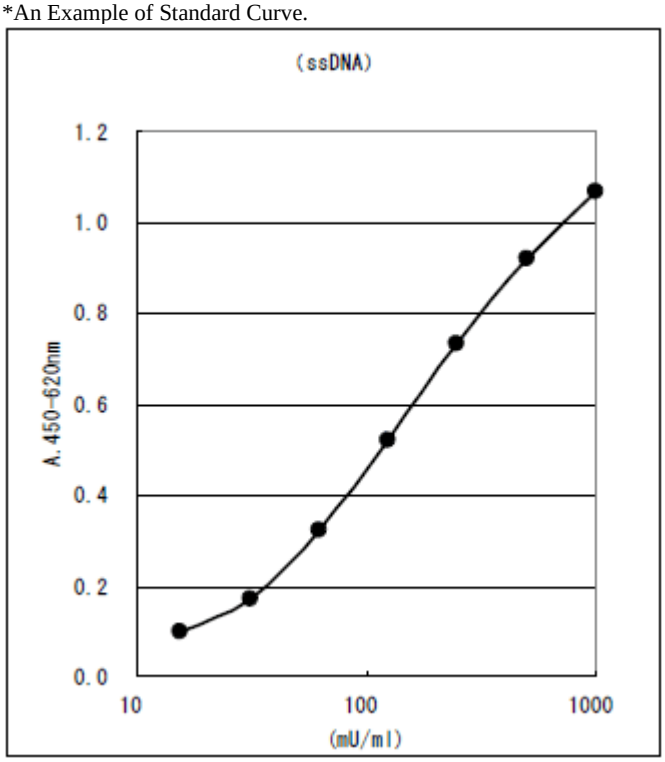
Term of validation

Six months from manufacturing day.

Precautions

(1) Please read this manual carefully prior use.
(2) This kit has been designed for research use only.
(3) Do not use the reagents with different lot numbers together.
(4) Be careful not to spill the chromogenic substrate reagent and the acid stop solution on the skin or mucous membrane.

*An Example of Standard Curve.



Calculation of concentration

(1) Prepare a standard curve using semi-logarithmic or bi-logarithmic section paper by plotting absorbance* (Y-axis) against standard concentration (mU/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 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 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. If you use logarithm transformation for concentration and also absorbance, the fitness of the 3rd order regression will be improved.

Statements and Precautions as to Our Kits

* This assay kit should be used only for research works.
* The reagent solutions of the kit should be used principally immediately after dilution. Otherwise, keep them in a dark place at 2-8C,and use them within 5 days.
* 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 are preserved for some period.
* Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
* Do not dry the assay plate to avoid denaturation of the coated antibody or antigen.
* The reaction time should be counted from the onset of reagent pipetting.
* Prepare the standard curve in every assay.(For KIT with standard solution.)
* Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
* Preservation condition for the kit should be strictly kept.

* Be careful not to allow the reagent solutions of the kit to contact to skin and mucus. Especially treat the stopping solution very carefully because it contains sulfuric acid.
* HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper should be avoided from contacting to any metal.
* In treating assay samples of animal origin, be careful for possible biohazards.

Former page Page top

Shibayagi Co. Ltd. 1062-1, Ishihara, Shibukawa, Gunma Pref., 377-0007, JAPAN
TEL.+81-279-25-0279 FAX.+81-279-23-0313