SHIBAYAGI	Mouse-IgG Rheumatoid Factor ELISA Kit	Research Reagent

Please, read carefully this instruction before assay.

[Merit of the kit]

This assay kit can measure mouse IgG-type rheumatoid factor,

(1) Quickly (4.5 hours),
(2) With a small volume of sample (1-5 μl),
(3) Promptly and easily with all reagents provided as solution,
(4) With high reproducibility.

[Reagents]

A:	Mouse IgG Fc coated plate	96well(8x12)	x1
B:	Standard Mouse-antibody solution(10000mU/ml)*	100μl	x1
C:	HRP-conjugated anti-mouse IgG	20μl	x1
D:	Chromogenic substrate reagent(TMB)	12ml	x1
E:	Reaction stopper(1M H_2SO_4)	12ml	x1
F:	Buffer solution	60ml	x1
G:	Concentrated washing buffer(10x)	100ml	x1

* The units may differ among lots.

[Required but not delivered]

(1) Micropitette (1-1000μl)

(2) Microplate washing apparatus (microplate washer, shaker, wash bottler, etc.)

(3) Microplate reader

[Perparation of Ragent Solutions]

(1) Washing buffer : Prepare by diluting concenterated washing buffer to 1:10 with distilled water.

(2) HRP-conjugated antibody solution : Prepare by diluting the concentrated solution to 1:2,000 with assay buffer.

(3) Other reagents can be used undiluted.

(4) Use all the reagent solutions of the Kit after getting back to room temperature.

[Dilution of Assay Smples and Preparation of the Standard Antibody Solution] (We show an example)

(1) Assay samples : Dilute to 1:51, 1:101, 1:201 with the assay buffer.

(2) Standard antibody solutions: Prepare std 7 by mixing 100μl of attached original std soluiotn and 700μl buffer. Then prepare std 6 by mixing 250μl of std 7 and 250μl buffer, and so forth until std 1 by serial dilution.

	Std 7	Std 6	Std 5	Std 4	Std 3	Std 2	Std 1	Std 0
Potency (mU/ml)	1,000	500	250	125	62.5	31.3	15.6	0
Standard solution(μl)	100	250	250	250	250	250	250	0
Assay buffer(μl)	700	250	250	250	250	250	250	250

[Assay Procedure]

(1) Wash the assay plate 3 times with the washing buffer by filling the wells with the buffer and discarding. Thereafter, place the plate upsinde-down on the paper towel for a while to remove 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 2 hour 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. Thereafter, place the plate upside-down on the paper towel for a while to remove excess buffer.

(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 2 hour 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. Thereafter, place the plate upside-down on the paper towel for while to remove excess buffer.

(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).

Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.

[Calculation of assay value]

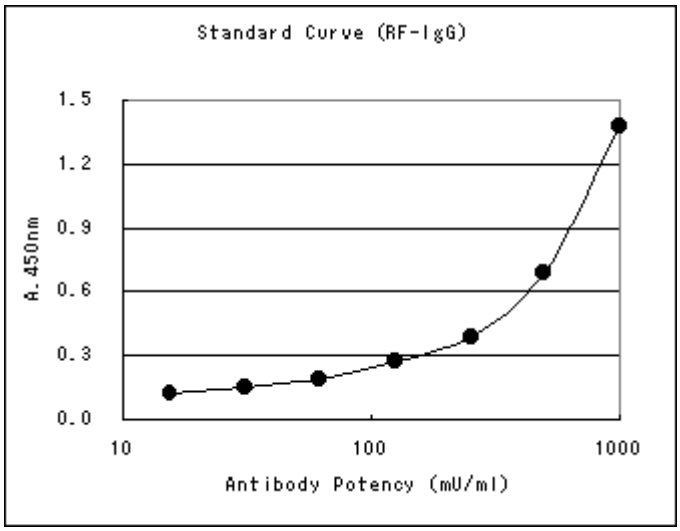
(1) Prepare a standard curve using hemi-logarithmic section paper with Y axis as 450nm absorbance (or 450nm absorbance-620nm absorbance), and X axis as logarithmic concentration of standard antibody concentration.

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

(3) 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 antibody level in the sample can be obtained by (assay value reading from the standard curve x dilution factor).

(4) We recommend you to confirm the linearity of the assay values after several dilution for a sample.

[Standard Curve (an example)]



[Summary of Assay Procedure]

Antigen-coated plate
Washing
| + Standard antibody solution or diluted sample; 100µl
Shaking , Reaction at room temp.(20 - 25C) for 2hr
|
Washing
| + HRP-conjugated antibody; 100µl
Shaking , Reaction at room temp.(20 - 25C) for 2hr
|
Washing

| + Chromogenic substrate (TMB) reagent solutin; 100µl
Shaking , Reaction at room temp.(20 - 25C) for 20min
| + Reaction stopper; 100µl
Shaking , Measurement of absorbance(450nm) (Sub 620nm)

[Assay Validation]

1. Assay range
Absorbance range corresponding to standard concentration 15.6 to 1000mU/ml is 0.05 to 2.5.
2. Specificity
As anti-mouse IgG type antibody is labeled with HRP, crossreactivity to IgM is lower than
ELISA background.
3. Assay precision
Within assay C.V. (n=30) is 6.9%
4. Reproducibility
Between assay C.V. (n=30, 3 days) is 8.7%

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

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