Cat. # MK136

For Research Use

TaKaRa

Human IgG EIA Kit

Product Manual

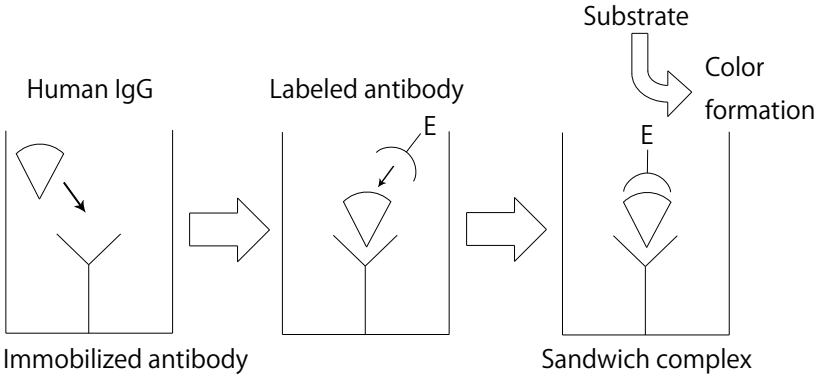
v202003Da

Table of Contents

I. Description

γ -Globulins are plasma proteins known as immunoglobulins (Ig) for their roles in the immune response. There are five types of immunoglobulins: IgG, IgA, IgM, IgD, and IgE. One of the immunoglobulins, IgG, is produced by plasma cells differentiated from activated B lymphocytes and functions as many roles; as various types of immune antibodies, as neutralizing antibodies that destroy infectiveness, and as opsonizing antibodies that help capture bacteria. IgG is a placenta-patency plasma protein produced by lymph nodes, spleen, bone marrow, thymus, small intestinal mucosa, and respiratory tract mucosa, among other tissues. Since IgG is produced as the body's response to stimulations by bacteria, viruses, drugs, and tissue antigens, measuring the blood level of IgG can reveal any hyperresponse to antigen stimulations or anomalies at IgG-producing sites, among other disorders. The presence of high IgG levels has been linked to polyclonal hypergammaglobulinemia, collagen disease, asymptomatic M-proteinemia, chronic infections, myeloma, and IgG multiple myeloma, and other pathological conditions and diseases. A low IgG level, on the other hand, has been known to associate with disorders such as agammaglobulinemia or hypogammaglobulinemia, severe immunodeficiency, and nephrosis syndrome.
Human IgG EIA uses a human IgG-specific monoclonal antibody. It can be used as a simple tool to monitor IgG levels in human blood and body fluids, among other purposes.

II. Principle



III. Components

(1) Antibody Coated Microtiterplate — 1 plate
Anti-human IgG monoclonal antibody coated plate
(96 wells: 8 wells x 12 strips)

(2) Antibody - POD Conjugate — for 11 ml
Peroxidase-labeled anti-human IgG monoclonal antibody (lyophilized)

(3) Standard (Lyophilized) — for 1 ml
Human IgG Fc region (Recombinant protein) 640 ng*
＊ Amount of human IgG converted from that of the recombinant protein.

(4) Sample Diluent — 11 ml x 2
25% BlockAce in PBS containing a preservative

(5) Substrate Solution (TMBZ) — 12 ml
3,3′,5,5′-Tetramethylbenzidine solution

IV. Materials Required but not Provided

- Wash and Stop solution for ELISA without Sulfuric Acid (Cat. #MK021)
  Contains wash solution (10X PBS, 50 ml x 5 tubes; Tween 20, 3 ml) and reaction stop solution (60 ml).
  ＊ This product is a stop solution for peroxidase reactions without 1N sulfuric acid.
  ＊ 1N sulfuric acid can be used as a stop solution. Handle 1N sulfuric acid with caution.
- Pipette, micropipette, and tips
- Microplate reader (capable of measuring absorbance of up to 3.5 when set to 450 nm)

V. Storage 4℃

VI. Intended Purpose

In vitro enzyme immunoassay (EIA) for quantitative determination of human igG in human blood and body fluid samples

VII. Protocol

1. Sample

- Store samples at 2 - 10℃ or in a freezer if the samples will not be assayed within 12 hours after collected.
- For sample dilutions, refer to the standard curve presented later in this manual. Dilute samples expected to contain a high level of IgG with (4) Sample Diluent.
- When human blood sample is used, the recommended dilution is $>10^5$ fold as a standard.

2. Reagent preparation

- Antibody plate (1) Antibody Coated Microtiterplate
  Return the unopened plate to room temperature in its package before use.

- POD-labeled antibody solution
  Reconstitute (2) Antibody - POD conjugate with 11 ml of distilled water.
  Once reconstituted, it is stable for up to 1 week at 4℃. For longer storage, freeze at -20℃; under this condition, it is stable for up to 1 month. Once thawed, it should not be returned to frozen storage.

- Human IgG standard solution
  Add 1 ml of distilled water to reconstitute (3) Standard, and prepare a human IgG standard solution (640 ng/ml). The concentration of standard is shown as human IgG. Dilute with (4) Sample Diluent before use to prepare fresh serial dilutions (standard solutions at concentrations of 640, 320, 160, 80, 20, and 10 ng/ml). Use (4) Sample Diluent as the 0-concentration standard. The human IgG standard solution (640 ng/ml) is stable for up to 1 week after preparation when stored at 4℃, or for up to 1 month at -20℃.

- Substrate solution (5) Substrate Solution (TMBZ)
  Return the substrate solution to room temperature before use. It is supplied ready to use in reactions. Check before use that it has not developed a dark blue color.
  A reaction with metal ions will result in coloration; make sure it is not contaminated with any tap water.
  If the substrate solution will be used for several reactions, divide it into aliquots of the required volume in advance.

- Stop solution
  Use the Stop solution included in Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021) directly.
  ＊ Because this is highly viscous, mix well using a plate mixer after its introduction.

- PBS with 0.1% Tween 20 for washing
  Dilute the 10X PBS included in Wash and Stop solution for ELISA without Sulfuric Acid (Cat. #MK021) 10 fold with distilled water, and then add Tween 20 to a final concentration of 0.1%.
  For 96 reactions performed with this kit, 300 ml of washing solution is required.

3. Procedure

Assay samples in duplicate.
Return each reagent in the kit and samples to room temperature and make sure solutions are mixed uniformly without creating bubbles before use.

(1) Using micropipettes, add 100 μl of each concentration of human IgG standard solution and each sample per well to 2 strips and allow to react for 1 hour at room temperature (20 - 30℃). Prepare reagents and samples in a separate 96 well plate in advance so that they can be added to the antibody-plate quickly (within 5 minutes) using an 8-channel pipette or something similar. In order to provide a higher reliability in the results, it is recommended to place serial dilutions of the standard solution in the 1st and 12th rows.
Make sure to perform this reaction at room temperature (20 - 30℃), because the incubation at 37℃ may compromise antigenicity. [First reaction]

(2) Discard reaction mixtures, and wash 3 times with 0.1% Tween 20-containing PBS. Then add 100 μl of the labeled antibody solution per well using an 8-channel pipette or something similar and allow to react for 1 hour at room temperature (20 - 30℃). [Second reaction]

(3) Discard reaction mixtures, and wash 4 times with 0.1% Tween 20-containing PBS. Then add 100 μl of (5) Substrate Solution (TMBZ) per well and allow to react for 10 - 15 minutes at room temperature (20 - 30℃). [Third reaction]

(4) Add 100 μl of (6) Stop Solution to each well in the same order that (5) Substrate Solution (TMBZ) was added to stop the reaction. Then mix well.

(5) Use distilled water as control to perform zero adjustment and measure absorbance at 450 nm. The color is stable for up to an hour after reaction is terminated.

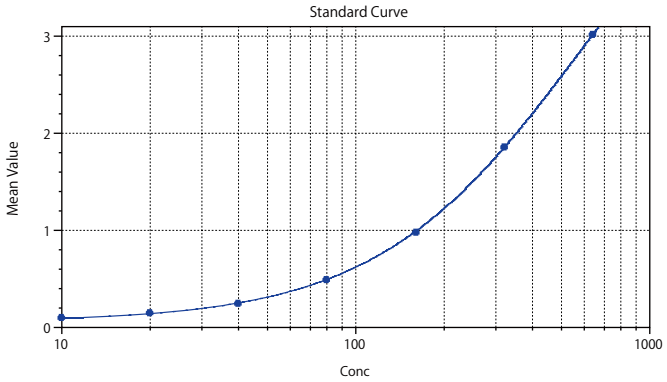
(6) Plot a standard curve based on the results obtained from the standard solutions (with concentration at x-axis and absorbance at y-axis). Determine the Human IgG concentration of sample by referring to the curve for the corresponding concentrations of human IgG based on the absorbance of samples.

VIII. Performance

1. Typical standard curve

The following shows a typical standard curve of this kit as an example.
The standard curve for calculation needs to be established in each assay.

Assay sensitivity : 10.0 ng/ml
Curve Fit : 4 - Parameter Corr. Coeff : -1.00
$y = (A - D) / (1 + (x / C)^B) + D$
A = 0.0526 B = 1.24 C = 596. D = 5.72



Human IgG (ng/ml)	640.0	320.0	160.0	80.0	40.0	20.0	10.0	0.0
A_{450}	3.009	1.853	0.977	0.489	0.250	0.144	0.092	0.043

2. Reproducibility

<Intra-assay reproducibility (n=16)>

The reproducibility test was performed with 16 replicates, using 3 different concentrations of control prepared from dilutions of human serum.

Sample	Mean (ng/ml)	CV (%)
Control A	473.5	4.8
Control B	165.8	5.0
Control C	30.5	2.9

<Inter-assay reproducibility test (n=3)>

The reproducibility test was performed with triplicates, by assaying 3 different concentrations of control over 3 days.

Sample	Mean (ng/ml)	CV (%)
Control A	448.2	4.0
Control B	158.5	4.1
Control C	29.2	6.7

3. Recovery test

Equal volumes of samples in different concentrations were combined and assayed. The assay result of each mixture was compared with the theoretical value to determine the recovery rate.

Sample A	Sample B	A+B (Theoretical Result)	A+B (Assay Result)	Recovery Rate (%)
6.9	2.0	4.5	4.1	92
21.5	15.8	18.6	20.0	107
38.0	15.8	26.9	25.2	94
58.1	15.8	36.9	40.5	110
21.5	123.2	72.4	63.1	87
150.0	30.9	75.2	90.5	83
181.4	15.8	98.6	99.7	101
150.0	72.1	109.1	111.0	98
181.4	38.0	109.7	125.2	114
58.1	181.4	119.7	130.5	109
126.0	123.2	124.6	105.8	85
231.6	58.1	144.8	144.2	100
181.4	123.2	152.3	140.6	92
231.6	126.0	178.8	183.3	103
233.6	72.1	194.0	152.8	127
382.0	15.8	198.9	239.9	121
231.6	181.4	206.5	210.4	102
382.0	58.1	220.0	258.7	118
382.0	123.2	252.6	260.7	103
382.0	231.6	306.8	340.2	111

Unit: ng/ml

4. Reactivity against human IgG subclasses

Purified myeloma-derived IgG samples prepared at a concentration of 500 ng/ml were used to study the reactvity against each subclass of IgG.

Human Ig	IgG1 κ	IgG1 λ	IgG2 κ	IgG2 λ	IgG3 κ	IgG3 λ	IgG4 κ	IgG4 λ
A_{450}	2.227	1.611	1.254	0.714	0.263	0.043	0.388	0.171

5. Cross reactivity with serum samples from various species
Cross reactions with antibodies in serum from various species were studied.

STD (ng/ml)	Serum Species (Dilution Ratio)			STD (ng/ml)
	1 x 10^2 1 x 10^5 2 x 10^5	1 x 10^2 1 x 10^5 2 x 10^5	1 x 10^2 1 x 10^5 2 x 10^5	
640	Human donor No. 1	Horse (male)	Rabbit	640
320	Human donor No. 2	Horse (female)	Chicken	320
160	Human donor No. 3	Porcine	Goose	160
80	Human donor No. 4	Human (pooled serum)	Turkey	80
40	Human donor No. 5	Dog	Domestic duck	40
20	Human donor No. 6	Guinea Pig	Cynomolgus No. 1	20
10	Bovine	Rat	Cynomolgus No. 2	10
0	Goat	Mouse	Cynomolgus No. 3	0

A_{450} absorbance

2.979	3.858 0.711 0.293	0.045 0.043 0.042	0.039 0.041 0.041	3.029
1.930	3.742 0.629 0.228	0.045 0.044 0.039	0.039 0.040 0.040	1.919
1.079	3.808 0.984 0.415	0.045 0.043 0.040	0.039 0.039 0.040	1.077
0.565	3.093 0.500 0.262	3.578 0.476 0.226	0.035 0.040 0.038	0.560
0.313	3.804 0.988 0.509	0.042 0.042 0.042	0.043 0.038 0.042	0.299
0.187	3.798 0.838 0.367	0.043 0.041 0.041	0.047 0.042 0.040	0.182
0.109	0.041 0.042 0.044	0.045 0.046 0.049	0.044 0.041 0.040	0.105
0.048	0.042 0.045 0.046	0.044 0.043 0.043	0.048 0.042 0.037	0.040

No cross reaction was detected at 100-fold dilution with animal serum samples.

6. Assay example: human urine sample

	Dilution ratio			Dilution ratio	
	X 11	X 110		X 11	X 110
No. 1	384	ND	No. 6	114	ND
No. 2	22	ND	No. 7	142	ND
No. 3	536	ND	No. 8	140	ND
No. 4	484	ND	No. 9	130	ND
No. 5	78	ND	No.10	104	ND

Measured value (ng/ml), N = 2, ND = Below detection limit

Ten human urine samples were assayed and the results showed that assays can be performed on human urine samples diluted up to 11 - fold.

IX. Notes

1. Do not mix-use kits or reagents from different lots.
2. Do not expose reagents to strong light during storage or reactions.
3. Use metal-free pipettes when handling (5) Substrate Solution (TMBZ).
4. Pay attention to prevent (5) Substrate Solution (TMBZ) from contacting hands or mucous membranes.
5. Do not use (5) Substrate Solution (TMBZ) that has developed color.
6. Each reaction varies depending on time and temperature. Therefore, a new standard curve must be established for each assay.
7. Handle blood samples with great care.