（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K812-M

Specification：48T (38 samples)/96T (86 samples)

Measuring instrument：Microplate reader(560-580 nm)

Detection range：0.84-40.00 mg/mL

Elabscience® Human Immunoglobulin G (IgG) Turbidimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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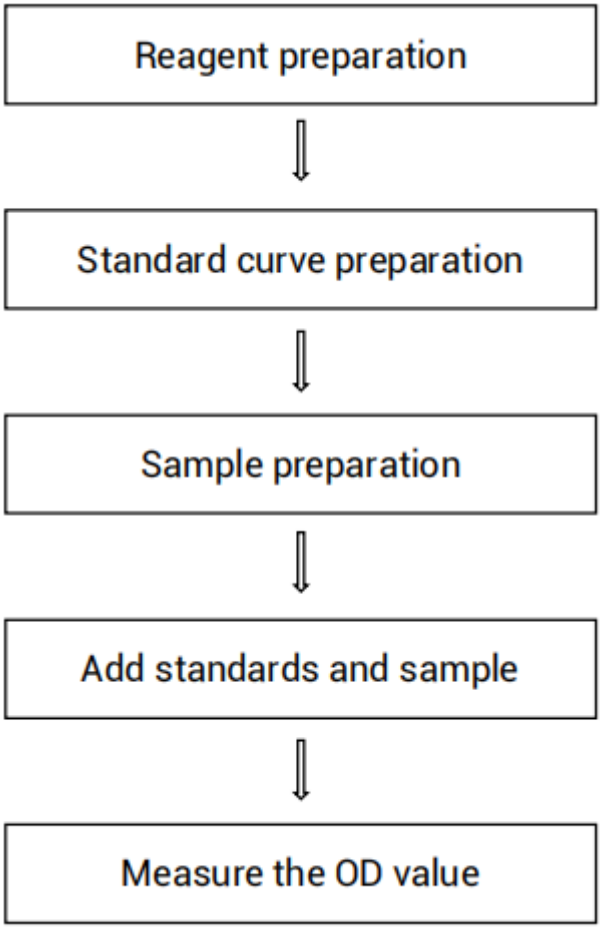
Assay summary



Intended use

This kit can be used to measure human immunoglobulin G (IgG) content in human plasma and human serum samples.

Detection principle

The quantification of immunoglobulin G (IgG) in serum is of great significance for the diagnosis of primary or secondary immune deficiencies, the monitoring of immunoglobulin therapy, and the clinical course of multiple myeloma.

The detection principle of this kit: the antigen-antibody reaction between human IgG and IgG antibodies, which forms immune complexes and causes turbidity in the reaction system. This turbidity results in an absorption peak at 570 nm, and the concentration is directly proportional to the absorbance.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	12.5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Agent	4.5 mL × 1 vial	9 mL× 1 vial	2-8°C, 12 months, shading light
Reagent 3	40 mg/mL Standard	0.05 mL × 1 vial	0.1 mL × 1 vial	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small l volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (560-580 nm, optimum wavelength: 570 nm), Incubator

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of standard curve：

Dilute 40 mg/mL standard solution with PBS(0.01 M，pH 7.4) to a serial concentration, the recommended dilution gradient is as follows: 0, 5, 10, 20, 40, mg/mL. Reference is as follows:

Item	①	②	③	④	⑤
Concentration (mg/mL)	**0**	**5**	**10**	**20**	**40**
40 mg/mL standard (μL)	0	2.5	5	10	20
PBS(0.01 M，pH 7.4) (μL)	20	17.5	15	10	0

Note: The diluted standard can be stored at 4°C for a month.

Sample preparation

① Sample preparation

Serum and plasma samples: detect directly.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human plasma	1

Note: The diluent is PBS(0.01 M，pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Read the values immediately after react exactly 5 min, the sample measurement should be conducted in no more than 30 wells at a time.

Operating steps

① Standard well: Add 2 μL of standard solution with different concentrations into the wells.
Sample well: Add 2 μL of sample into the wells.

② Add 225 μL of buffer solution into the wells.

③ Mix fully for 5 s with microplate reader and incubate at 37°C for 5 min. Measure the OD value of each well at 570 nm as A_1.

④ Add 75 μL of chromogenic agent into each well.

⑤ Mix fully for 5 s with microplate reader and incubate at 37°C for 5 min. Measure the OD value of each well at 570 nm as A_2, $\Delta A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as x-axis and y-axis respectively. Create the standard curve (**y=ax^2+ bx +C**) with graph software (or EXCEL).

The sample:

Serum and plasma samples:

$$\underset{(mg/mL)}{\text{IgG content}} = (a \times k^2 + b \times k + c) \times f$$

[Note]

k: $\Delta A_{sample} - \Delta A_{blank}$.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	8.27	12.12	16.60
%CV	5.7	4.6	5.5

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	8.27	12.12	16.60
%CV	8.5	7.1	9.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/mL)	5.22	7.83	10.44
Observed Conc. (mg/mL)	4.99	8.08	9.96
Recovery rate (%)	96	103	95

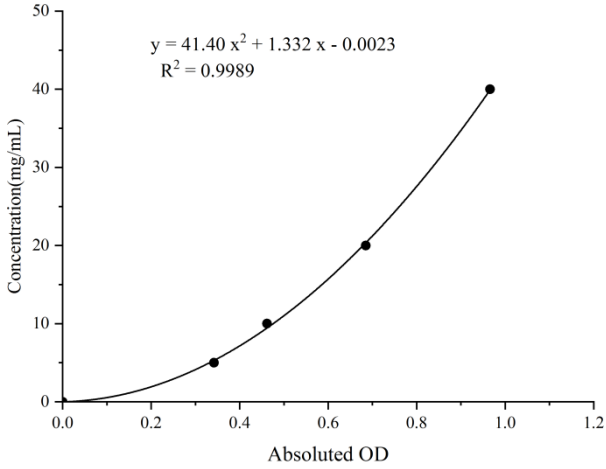
Sensitivity

The analytical sensitivity of the assay is 0.84 mg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mg/mL)	0	5	10	20	40
A_1 value	0.040	0.039	0.040	0.043	0.043
	0.041	0.041	0.042	0.043	0.043
A_2 value	0.311	0.649	0.780	0.984	1.283
	0.274	0.618	0.729	0.976	1.238
Average ΔA value	0.252	0.594	0.714	0.937	1.218
Absolute ΔA value	0.000	0.342	0.462	0.685	0.966



Appendix Π Example Analysis

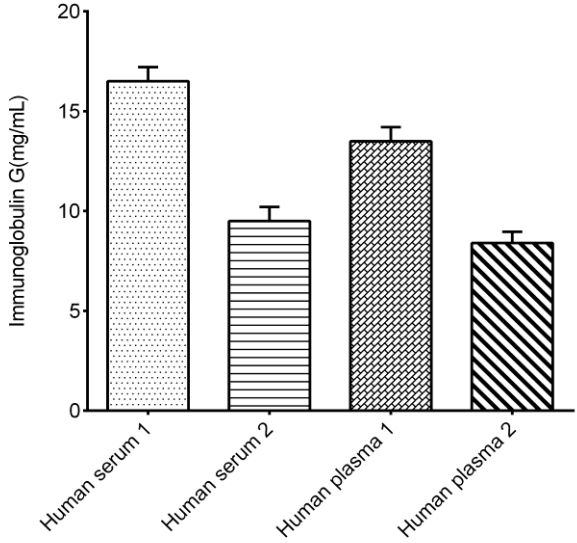
Example analysis：

Take 2 μL of human serum, and carry the assay according to the operation steps. The results are as follows:

standard curve: $y = 41.40 x^2 + 1.332 x - 0.0023$, the ΔA value of the sample well is 0.735, the ΔA value of the blank well is 0.252, and the calculation result is:

$$\text{IgG content (mg/mL)} = 41.40 \times (0.735 - 0.252) \times (0.735 - 0.252) + 1.332 \times (0.735 - 0.252) - 0.0023 = 10.30 \text{ mg/mL}$$

Detect human serum, human plasma, according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.