Human IgG (Total) ELISA Kit

E22-HC124

Catalog No.:E22-HC124.48, 48 tests; E22-HC124.96, 96 tests

This product is made of raw materials from world famous manufacturers and manufactured by professional in vitro diagnostic reagent production technology. It is only for scientific research. This product can detect the concentration of natural and recombinant human IgG in serum, plasma, cell culture supernatant, lavage fluid, urine, amniotic fluid, ascites, cerebrospinal fluid, pleural effusion, tissue homogenate and other types of samples. Please consult for specific samples. Please read the instructions carefully and check the reagent composition before use. For specific samples, please consult.Please read the instructions carefully and check reagent components before use.

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION: IgG(Immunoglobulin G) is a kind of antibody. It is composed of four peptide chains -- two identical heavy chains and two identical light chains, forming a typical Y-shaped antibody form. Each IgG has two antigen-binding sites. IgG is synthesized and secreted by B cells in plasma. It accounts for 75% of the immunoglobulin content in human serum and is the highest proportion of antibody type in peripheral circulation. IgG has four subtypes, namely IgG1, IgG2, IgG3 and IgG4. The main function of IgG is to protect the body from infection by pathogenic microorganisms such as bacteria, fungi and mycoplasma. IgG achieves this function through a variety of immune mechanisms: IgG binds to pathogens and aggregates to block their movement; IgG binds to the surface of pathogens so that they can be recognized and taken up by phagocytes. IgG can activate the classical complement pathway and finally dissolve the pathogen. IgG also binds to toxins produced by pathogens, thereby neutralizing their toxic effects. IgG plays an important role in antibody-dependent cell-mediated cytotoxicity (ADCC). In addition, IgG is also associated with type II and type III hypersensitivity reactions. IgG is produced 24-48 h after antigen stimulation.

PRINCIPLE OF THE ASSAY:

The anti-human IgG(Total) mab is coated on the HRP-plate. IgG(Total) in the specimen and standard will bind to the mab and the free component will be washed away. When HRP-conjugated antibody is added,

anti-human IgG(Total) antibody is combined with human IgG(Total) bound to the monoclonal antibody to form an immune complex, and the free component is washed away. Add the chromogenic substrate. If IgG(Total) is present in the reaction well, horseradish peroxidase will make the colorless chromogenic agent appear blue, and add the stop solution to turn yellow. The OD value was measured at 450nm, and the concentration of IgG(Total) was positively proportional to the OD450 value. The concentration of IgG(Total) in the specimen could be calculated by drawing the standard curve.

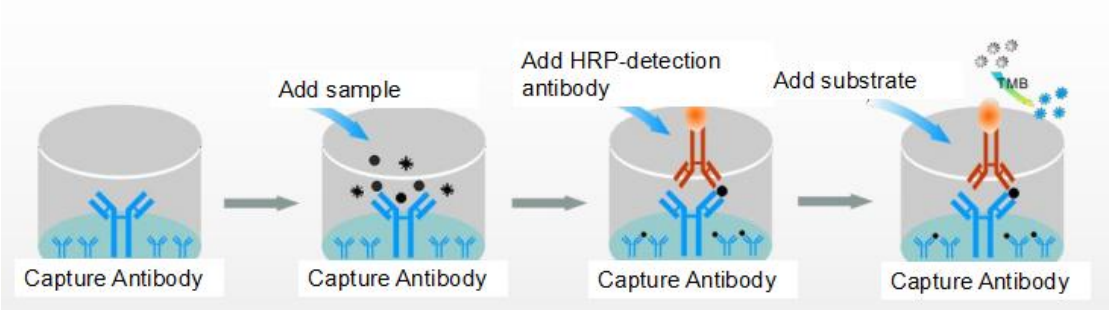


Figure 1: Schematic diagram of the assay

REAGENTS

1. Aluminium pouches with a Microwell Plate coated with monoclonal antibody to Human IgG (8X12)

2.2 vialsHuman IgGalpha Standard lyophilized, 1000 pg/ml upon reconstitution

3.2 vials concentrated Biotin-Conjugate anti-Human IgG alpha antibody

4.2 vials Streptavidin-HRP solution,

5.1 bottle Standard /sample Diluent

6.1 bottle Biotin-Conjugate antibody Diluent

7.1 bottle Streptavidin-HRP Diluent

8.1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)

9.1 vial Substrate Solution

10.1 vial Stop Solution

11.4 pieces Adhesive Films

12.package insert

NOTE: [96 Tests]

STORAGE

Table 1: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.**</td></tr>
<tr><td>Concentrated Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP solution</td></tr>
<tr><td>Biotin-Conjugate antibody Diluent</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20°C. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.**</td></tr>
</table>

**Provided this is within the expiration date of the kit.

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).

2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.

3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1.Store kit regents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).

2.Please perform simple centrifugation to collect the liquid before use.

3.To avoid cross contamination, please use disposable pipette tips.

4.The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.

5.Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent.

Mix all reagents and samples well before use.

6.After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.

7.Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.

8.Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.

9.Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.

10.Avoid microtiter plates drying during the operation.

11.Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

12.Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.

13.This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1.**Cell Culture Supernates** - Remove particulates by centrifugation.

2.**Serum -** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.

3.**Plasma -** Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.

4.Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

5.Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).

Note: The normal rat serum or plasma samples are suggested to make a 1:2 dilution.

REAGENT PREPARATION

1. Remove the kit from the refrigerator 20 minutes in advance to balance to room temperature.

2. Dilute 20× washing solution with double distilled water to 1× working solution.Return unused to 4℃.

3. Standard: Add 0.4 ml of standard & specimen common diluent to lyophilized standard, then leave Leave for 15 minutes and mix gently (8000 pg/ml) after it is fully dissolved.Then dilute as needed.(It is recommended to use the following concentrations for standard curves: 8000, 4000, 2000, 1000, 500, 250, 125, 0 pg/ml).0 pg/ml is the blank hole.Redissolve standard stock solution (8000 pg/ml) If not used up, discard.

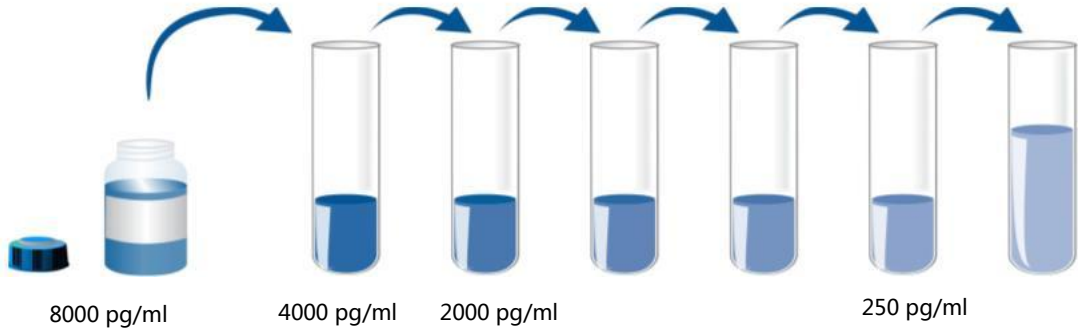


Figure 2: Take 500 ul/ tube as an example, redissolve standard mother liquor with standard & Specimen common diluent.The specific dilution method is shown in the figure below, or it can be diluted according to the actual dosage, for example 200 ul/tube.

4. Biotinylated antibody working solution: dilute 30× concentrated biotinylated antibody into 1× working solution with biotinylated antibody diluent 20 minutes before use according to the amount required for this test.Same-day use.If the amount of biotinylated antibody is insufficient due to the excessive number of experiments, biotinylated antibody can be purchased separately from Enjing Company.(Antibody lot number required)

5. Enzyme conjugate working liquid: according to the amount required by the test, 20 minutes before use, use. Enzyme conjugation dilution diluted 30× concentrated enzyme conjugation into 1× working solution.Same-day use.

The working solution should be used within one day after dilution.

GENERAL ELISA PROTOCOL

1.Prepare all reagents and working standards as directed in the previous sections.

2.Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.

3.Add 100uL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.

4.Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350uL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5.Add 100 uL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hours at 37℃.

6.Repeat the aspiration/wash as in step 3.

7.Add 100 uL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃ Avoid placing the plate in direct light.

8.Repeat the aspiration/wash as in step 3.

9.Add 100 uL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.

10.Add 100 uL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

11.Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length;610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

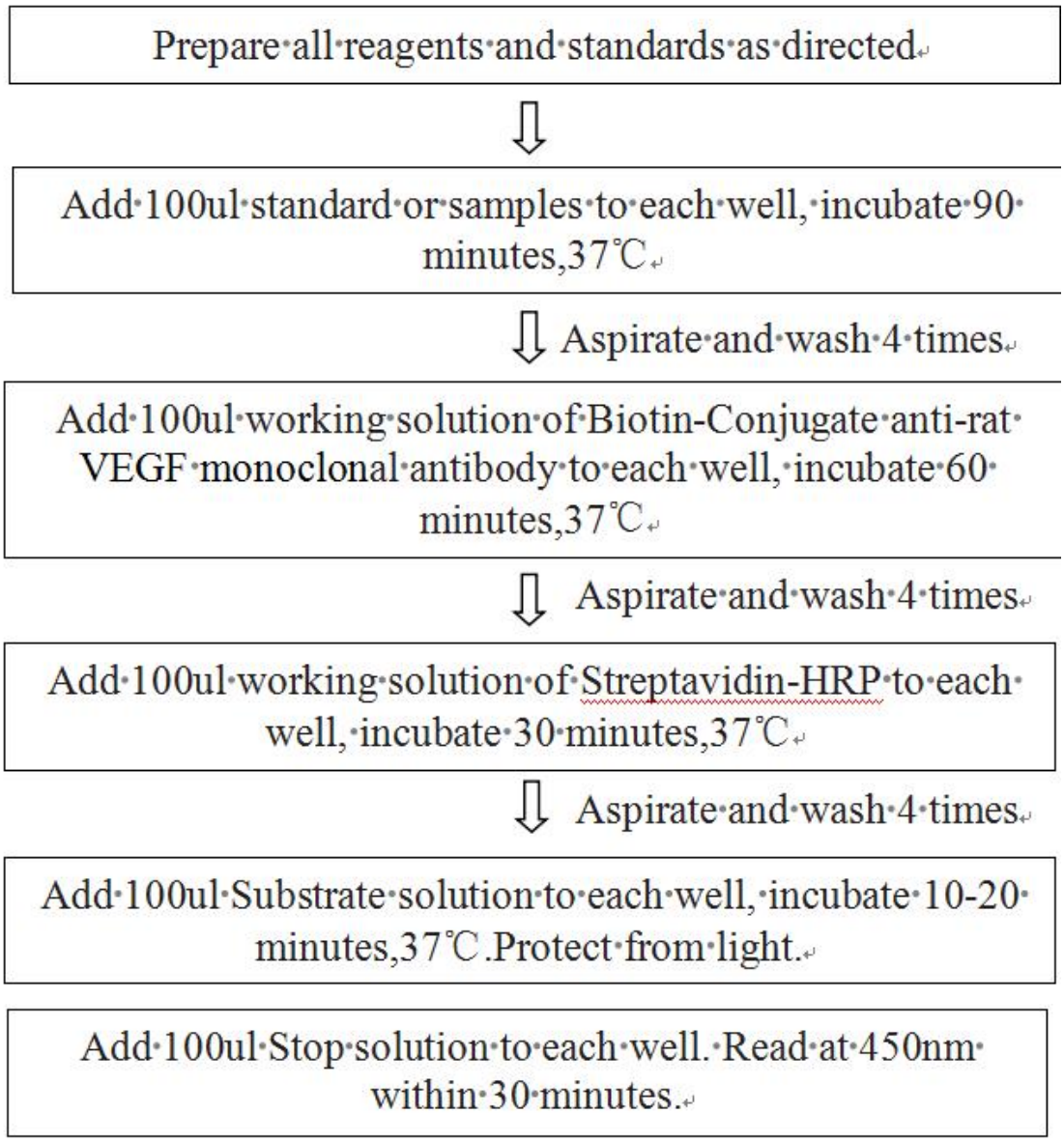


Figure 3: Assay procedure summary

TECHNICAL HINTS

1.When mixing or reconstituting protein solutions, always avoid foaming.

2.To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3.To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

4.Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

5.A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1.Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

2.Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

3.The data may be linearized by plotting the log of the IgGalpha concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

4.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Chart of data

Dose (ng/ml)	Raw Data		Average	Corrected
0	0.074	0.083	0.079	
0.78	0.162	0.18	0.171	0.092
1.56	0.241	0.246	0.244	0.165
3.13	0.381	0.372	0.377	0.298
6.25	0.662	0.614	0.638	0.559
12.5	1.077	1.142	1.110	1.031
25	1.849	1.817	1.833	1.754
50	2.956	2.979	2.968	2.889

Plot human IgG standard curve

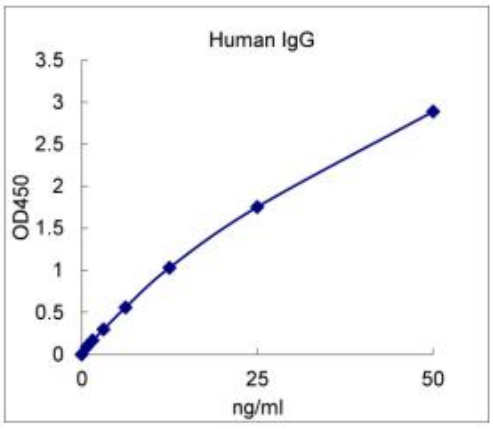


Figure 4: Representative standard curve for IgG alpha ELISA. IgGalpha was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

SENSITIVITY, SPECIFICITY AND REPEATABILITY

1.**REPEATABILITY:** The coefficient of variation of both intra-assay and inter-assay were less than 10%.

2.**SENSITIVITY:** The minimum detectable dose was 7pg/mL.

3.**SPECIFICITY:** This assay recognizes both natural and recombinant Human IgGalpha. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 3: Factors assayed for cross-reactivity

	In-plate repeatability			Interplate repeatability		
sample	1	2	3	1	2	3
Repeat the number	10	10	10	8	8	8
Average (pg/ml)	17.42	4.68	0.75	17.14	4.73	0.71
The standard deviation	0.649	0.255	0.044	0.638	0.226	0.055
Coefficient of variation (%)	3.7	5.4	5.9	3.7	4.8	7.7

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RELATED PRODUCTS

Table 4: Related products

Products name	Catalog number	size
Rat IL-2 ELISA Kit	E16RE0001	48T/96T
Rat IL-4 ELISA Kit	E16RE0004	48T/96T
Rat IL-6 ELISA Kit	E16RE0005	48T/96T
Rat IL-10 ELISA Kit	E16RE0007	48T/96T
Rat IFN-γ ELISA Kit	E16RE0002	48T/96T
Rat TNF-α ELISA Kit	E16RE0003	48T/96T
Rat MIP-2 ELISA Kit	E16RE0009	48T/96T