Human Immunoglobulin G (IgG) ELISA Kit

96 Well

Catalogue Number: NE01I0431

Valid Period: 6 months

For samples:

ELISA Kit for the quantitative Measurement of human IgG Residues in Protein Purification Process, and End-Product (purified fermentation broth, cell culture supernatant, etc.)

FOR RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. APPLICATION

This Human IgG ELISA kit is for research use only, it should not be used in clinical diagnostic procedures.

2. BACKGROUND

Immunoglobulin is a group of globulins with special chemical structure and immune function that exists in the body fluids and on the surface of lymphocytes, it is a material base for antibodies. The five major classes of immunoglobulin defined by their different structures and functions are IgG, IgM, IgA, IgD and IgE. Their molecular size, charge, amino acid composition and carbohydrate content are very uneven. Immunoglobulin molecule carries out dual tasks—binding to antigens and stimulating antibody production.

Immunoglobulin G (IgG) is the most abundant immunoglobulin in serum, it makes up 75% of the serum immunoglobulin levels. The average amount of IgG circulating in a healthy adult is 9.5~12.5mg/ml, 40～50% of which are distributed in serum, and the rest are distributed in tissues. Human IgG can be divided into IgG1, IgG2, IgG3, and IgG4 subclasses. They vary in the degree of binding abilities in the process of complement activation.

3. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the double antibody sandwich Enzyme Linked Immunosorbent Assay to detect the concentration of human IgG in samples. The microtiter plate has been pre-coated with goat anti-human antibody , standards or samples are then added to the microtiter plate wells and IgG if present, will bind to the antibody pre-coated wells under specific conditions. After washing, then a coupled biotin-conjugated detection goat anti-human antibody is added to the wells, under room temperature incubation, to form a precoated antibody-human IgG-detection antibody sandwich complex. After washing, coupled avidin conjugated Horseradish Peroxidase (HRP) is added to the wells, resulting in a cascade amplification effect. Then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP, and finally turns to yellow immediately after adding the stop solution. Color of TMB substrate positively correlated with total IgG bound in the initial steps. The color is measured by spectrophotometrically with

wavelength of 450nm. The concentration of human IgG in samples is then determined by comparing the O.D. of the samples to the standard curve.

4. KITS' ADVANCEMENT

1) High Specificity: Capture antibody and detection antibody respectively identify different epitopes of the antigen, which maximizes the specificity of the reaction. No cross-reaction exists between the homologous antigens.
2) High Sensitivity: Detection antibody conjugated biotin cross-link with polyvalent and high-affinity avidin to initiate cascade amplification to maximize the sensitivity.
3) High Stability: the experiment uses high-quality coated antibodies and antigens, and also applies the broad-spectrum protein stabilizers, and antibody pre-coated microplate treatment to increase the thermostability of the microplate, and reproducibility of results.
4) Optimal Diluent Buffer: Use a unique buffer optimized to purified stages' samples of recombinant protein to get rid of matrix interference. It is suitable for the quantification of purified products.

5. MATERIALS AND EQUIPMENTS (Note: Storage at 2-8℃)

	REAGENTS	SPECIFICATION	QUANTITY
1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -5ug/ml	20ul	1 vial
3	Detection antibody (100×)	150ul	1 vial
4	HRP-conjugated antibody(200×)	100ul	1 vial
5	Diluent Buffer (10×)	10ml	1 vial
6	TMB Substrates	10ml	1 vial (Avoid Light)
7	Stop Solution	10ml	1 vial
8	Wash Solution (100×)	10ml	1 vial
9	Plate Sealer		4 pieces
10	Instruction Manual		1

6. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) Precision pipettors and disposable tips to deliver 10-1000 µl.
2) A multi-channel pipette is desirable for large assays.
3) 1L sterilized deionized water or hyperpure water.
4) Sterilized EP Tubes
5) Absorbent Paper
6) Microplate reader capable of measuring absorbance at 450 nm.
7) High-speed Centrifuge
8) Automated Microplate Washer and Washing Bottle.
9) Incubator or Water bath (37°C).
10) Data analysis and graphing software.
11) Preparation of PBS: NaH_2PO_4 0.2g, $Na_2HPO_4 \cdot 12H_2O$ 2.9g, NaCl 8g， KCl 0.2g. Then dilute these to 1000ml with distilled water.

7. SPECIMEN COLLECTION AND STORAGE

Serum - Use a serum separator tube and allow samples to clot for 1-2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 1000×g (or 3000 rpm) for 15 minutes. Collect serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed 300-500mg before homogenization. Minced the tissues to small pieces and homogenized them in 500ul of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500×g (or 5000 rpm).Collect the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.

Cell lysates - Cells should be lysed according to the following directions.

1) Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
2) Wash cells three times in PBS. As for the collection of the samples, the amount of cells should be no less than 10^8 in 200ul PBS.
3) Cells were resuspended in PBS and subjected to ultrasonication for 3 times. Alternatively, freeze cells at -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.
4) Centrifuge at 1000×g (or 3000 rpm) for 15 minutes at 2-8°C to remove cellular debris.

Assay immediately or store samples at -20°C or -80°C.

Cell culture supernatants and other body fluids - Centrifuge cell culture media at 1000×g (or 3000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20°C or -80°C.

NOTE:

- Samples should be aliquoted and must be stored at -20° C (less than 3 months) or -80° C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8° C. Avoid repeated freeze-thaw cycles. Fresh samples without long time storage are recomended for the test. Otherwise, protein degradation and denaturalization may occur in those samples.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Samples containing a visible precipitate must be clarified prior to use in the assay.
- Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.
- Do not use heat-treated specimens.

8. PREPARATION BEFORE ASSAY

1) Please read through this manual carefully before using.
2) The frozen samples should be slowly restored to room temperature and then mix well, centrifuge the samples at 12000 rpm for 1 minute. Collect the supernatant for immediate assay.

3) Sample Dilution: Please predict the concentration before assaying. If values for these exceed the highest detection range of the kit, please use PBS to make the dilutions. It is recommended to set up several dilution factors for preliminary experiments, with a 10-fold dilution between adjacent concentrations.

NOTE:

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Influenced by the factors including low cell viability, small cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples, and lead to inaccurate results.
- The optimal pH value of the sample is between 7.0-7.4.

9. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30mins before using.
2) Wash Solution: Dilute 10 mL of Wash Solution concentrate (100×) with 990 mL of deionized water to prepare 1000 mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The 1× wash solution is stable for 2 weeks at 2-8°C.
3) The preparation of Diluent Buffer (1×): Dilute 10 mL of Diluent Buffer (10×) with 90 mL of deionized water to prepare 100 mL of Diluent Buffer (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

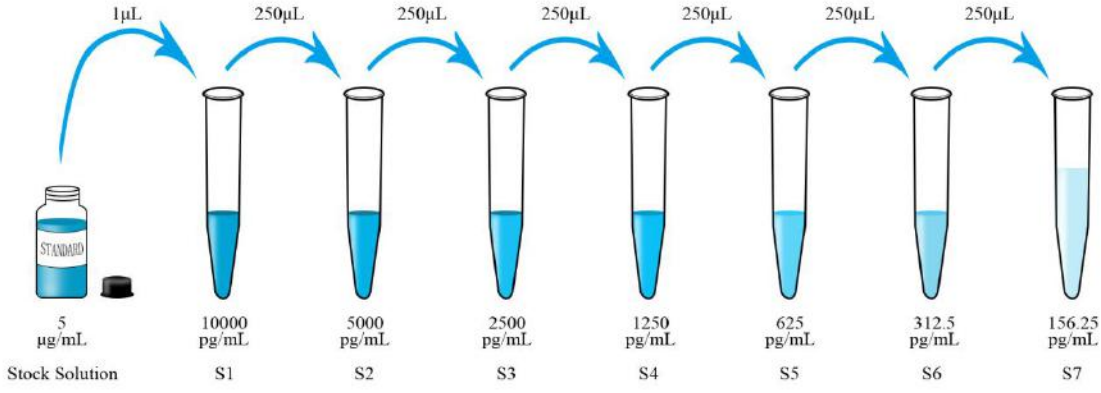
10. ASSAY PROCEDURES

1) Remove the microtiter plates from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean micropore plate frame, and start the experiment after the strips returned to room temperature.（Note: The microplate frame can be reused.）

2) Prepare Standards: When preparing the standards, label 7 tubes, S1-S7 and add a certain volume of diluent:S1 (499ul), S2 to S7 (each 250ul).

Add 1ul of the Standard (Stock Solution-5ug/ml) into the tube labeled as S1. Shake well and then pipette 250ul Standard solution (10ng/ml) from S1 to S2. And then produce a 2-fold dilution series until S7 . Secure the desired plate in the holder then add 100ul of diluted standards (S1-S7) or 100ul (diluted or undiluted) samples to appropriate wells. For the Blank Control test, we recommend add 100ul Diluent Buffer to the well.

NOTE: There are 7 points of diluted standards, S1:10000pg/ml, S2: 5000pg/ml, S3: 2500pg/ml, S4: 1250pg/ml, S5: 625pg/ml, S6: 312.5pg/ml, S7: 156.25pg/ml.



3) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at room temperature.

4) Automated Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle for standby.

- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no no moisture appears.
- Fill each well with 300ul Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times. Note: There should be no moisture appears in the fifth washing step.

5) Extract 100ul of detection antibody (100×), and add it into 10ml Diluent Buffer (1×) to reach its working concentration (1×) and mix gently. Add 100ul of above diluted detection antibody to each well, cover the plate with a sealer, and then incubate for 60 mins at room temperature. Repeat the wash process as above Step 4.
6) Extract 50ul HRP-Streptavidin (200×), and add it into 10ml Diluent Buffer (1×) to reach its working concentration(1×) and mix gently. Add 100ul of above diluted HRP-Streptavidin to each well,cover the plate with a sealer, and then incubate for 45 mins at room temperature. Repeat the wash process as above Step 4.
7) Add 100ul TMB substrate to each well. Put it on the oscillator to mix for 30s. Cover the plate with a sealer, incubate at 37℃ for 10 to 30 mins. (Avoid sunlight).

   Note: The coloring time varies in different experimental **conditions** (temperature, humidity, ect.).
8) Add 50ul of Stop Solution to each well to stop the reaction. Put it on the oscillator to mix well for 30s. Immediately run the microplate reader and conduct measurement at 450nm.

11. NOTES

1) **Sample Preparation**
   a) After collection, the samples should be aliquoted and stored at -20℃ (less than 3 months) or -80℃ (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8°C.
   b) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or

Vortex, centrifuge to remove flocculent insoluble substances before use.

c) If turbidity appears in the stock solution (Standard, Detection antibody and HRP-Streptavidin), mix gently or pipette up and down for several times.

d) It is recommended that all standards, controls and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

e) Do not use hyperlipidemia or hemolysis samples, which may interfere with ELISA and lead to inaccurate results.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

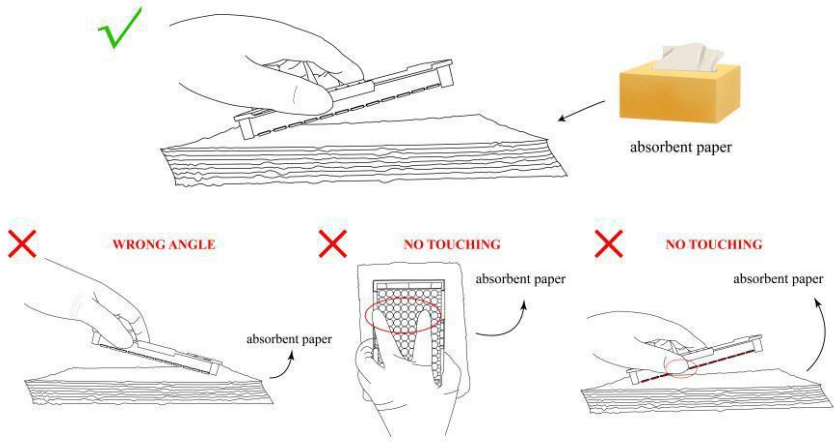
e) TMB is easily contaminated. Please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

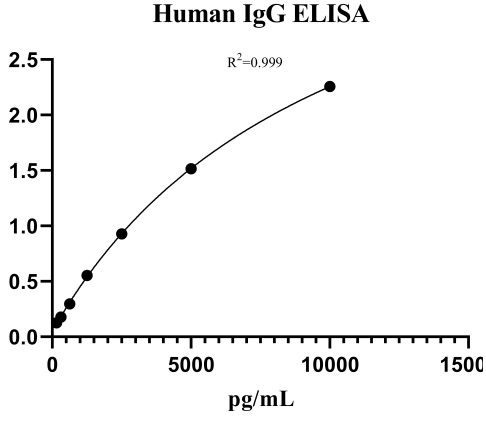
i) Schematic diagram of tapping the plate:



12. CALCULATION OF RESULTS

1) Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.
3) Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.
4) Standard curve for demonstration only.

APPENDIX 1 : EXAMPLE OF STANDARD CURVE



pg/ml	OD
10000.000	2.257
5000.000	1.515
2500.000	0.929
1250.000	0.553
625.000	0.296
312.500	0.179
156.250	0.126

13. QUALITY CONTROL

1) Intra Variation%: 2.7-3.14

2) Inter Variation%: 8.4-9.6

3) Recovery%: 88.77-113.22

4) Linearity:

Dilution Ratio	Range %	Average Linearity %
1	97.43-98.31	97.87
1:2	103.01-103.30	103.15
1:4	97.23-102.06	99.65
1:8	89.02-101.74	95.38
1:16	99.86-114.11	106.98
1:32	101.25-105.87	103.56
1:64	80.07-96.14	88.11

5) Sensitivity: 10.2 pg/ml

6) Specificity/Cross-reactivity:

Sample	Cross reactivity（%）
Mouse IgG	0.349
Bovine IgG	---
VERO HCP	---
293 HCP	---
MDCK HCP	---
OVA	---
chicken anti-SPA	---
TRF	---

7) Hook Capacity: This ELISA kit is applied to the three-step sandwich Enzyme Linked Immunosorbent Assay. Samples with high concentrated IgG will be effected by Hook Capacity. Please dilute the samples with PBS till it's within the detection range of the ELISA kit.

Samples' Dilution Ratio	OD
1	1.105

10	2.583
1000	3.596
100000	3.701
10000000	2.317
100000000	1.495
1000000000	0.301

8) Limitation: This ELISA kit is not suitable for the samples containing NaN3. NaN3 is a strong inhibitor for HRP that will decrease the concentration of the target protein.

14. CONTACT US

Address: 201318, Building 6, Lane 889, Ziping Road, Zhoupu Town, Pudong New Area, Shanghai, China

Website: www.bluegene.cc www.elisakit.cc

Tel: 400-882-6373/021--61106433/021--61106434/021--61106435/021--61106436

International Marketing Department:		
America	Tina	americas@bluegene.cc
Asia	Chris	asia@bluegene.cc
Australia	Chris	asia@bluegene.cc
Europe & Africa	Raina	europe-raina@bluegene.cc
Others	Cheng	sales@bluegene.cc
Technical Support Department:		
Menan	tech@bluegene.cc	