Datasheet for ABIN510001

IgG ELISA Kit

1 Validation

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Overview

Quantity:	96 tests
Target:	IgG
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.56 ng/mL - 100 ng/mL
Minimum Detection Limit:	1.56 ng/mL
Application:	ELISA

Product Details

Purpose:	This ELISA kit is a solid phase ELISA designed for quantitative determination of Immunoglobulin G.
Sample Type:	Cell Culture Supernatant, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	1.0 µg/mL
Characteristics:	The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of IgGin the sample, this IgGELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus IgGconcentration. The concentration of IgGin the samples is then determined by comparing the O. D. of the samples to the standard curve.

Product Details

Components:	• Microtiter plate (96 wells stripwell) - 1 • Enzyme conjugate - 1 vial • Standard A - 1 vial • Standard B - 1 vial • Standard C - 1 vial • Standard D - 1 vial • Standard E - 1 vial • Standard F - 1 vial • Substrate A - 1 vial • Substrate B - 1 vial • Stop solution - 1 vial • Wash solution - 1 vial • Balance solution - 1 vial • Instruction manual - 1
Material not included:	• Precision pipettors and disposable tips to deliver 10-1000 µL. A multi-channel pipette is desirable for large assays. • 100 mL and 1 L graduated cylinders. • Distilled or deionized water • Tubes to prepare sample dilutions. • Absorbent paper. • Microplate reader capable of measuring absorbance at 450 nm. • Centrifuge capable of 3000 x g. • Microplate washer or washing bottle. • Incubator (37 °C). • Data analysis and graphing software.

Target Details

Target:	IgG
Alternative Name:	Immunoglobulin G (IgG Products)
Target Type:	Antibody

Application Details

Application Notes:	• The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of samples used in the whole test. Please reserve sufficient amounts of samples in advance. • Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products.
- Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized 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 finally lead to wrong results.

Comment:

- It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time.
- The coefficient of determination of the standard curve should be higher or equal 0.95 and the highest O.D. should be more than 1.0.
- Cover or cap all kit components and store at 2-8°C when not in use.
- Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity.
- Samples should be collected in pyrogen/endotoxin-free tubes.
- Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
- Do not mix or interchange different reagent lots from various kit lots.
- Do not use reagents after the kit expiration date.
- Read absorbance immediately after adding the stop solution.
- 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.
- Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.

Information on standard material:

Different kits have different standards. For kits detecting proteisn or peptidse, the standards are recombinant proteins or synthetic peptides. For kits detecting small chemical compounds, the standards are synthetic chemical compounds. There are no standards extracted from natural

	resources. All of our reombinant proteins are expressed in E.coli. The standard are dissolved in PBS with 0.1 % proclin 300 and some other preservatives. Information on reagents: The STOP solution is 1M sulphuric acid. The wash buffer is 0.05 % Tween 20 in PBS, pH 7.4. The ELISA kit dose not contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME). Part of the reagents contain BSA. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	50 µL
Assay Time:	1.5 h
Plate:	Pre-coated
Reagent Preparation:	• **Samples** - Please predict the concentration before assaying. If concentrations are unknown or not within the detection range, a preliminary experiment is recommended to determine the optimal dilution. PBS (pH 7.0-7.2) or 0.9% physiological saline can be used as dilution buffer. • **Wash solution** - Dilute 10mL of wash solution concentrate (100×) with 990mL of deionized or distilled water to prepare 1000mL of wash solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have dissolved. The 1× wash solution is stable for 2 weeks at 2-8°C. Note: • Bring all kit components and samples to room temperature (20-25°C) before use. • Do not dilute other ready-to-use components.
Sample Collection:	• **Serum**: Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 1000 × g (or 3000rpm) for 15 minutes. Remove 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 100 × g (or 3000rpm) 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, thoroughly rinse tissues in ice-cold PBS (0.02mol/L, pH 7.0-7.2) to remove excess blood and weigh before homogenization. Mince the tissues into small pieces and homogenize them in a certain amount of PBS with a glass homogenizer on ice. Subject the resulting suspension to ultrasonication or to two freeze-thaw cycles to further break down cell membranes. After that, centrifuge for 15 minutes at 1500 × g (or 5000rpm). Remove 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 three times in PBS.
  - 3. Resuspend cells in PBS and subject to ultrasonication 3 times. Alternatively, freeze cells at -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.
  - 4. Centrifuge at 1000 × g (or 3000rpm) for 15 minutes at 2-8°C to remove cellular debris.
  - 5. 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 3000rpm) 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 (lower or equal 3 months) or -80°C (lower or equal 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.
- Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.
- 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.

Assay Procedure:	Prepare all Standards before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate. 1. Secure the desired number of coated wells in the holder then add 50 µL of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. 2. Add 50 µL of Conjugate to each well. Mix well. Complete mixing in this step is important. Cover and incubate for 1 hour at 37 °C. 3. Wash the Microtiter Plate using one of the specified methods indicated below: 4. Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with wash solution, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of FIVE washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly hen washing the plate to assure that all strips remain securely in frame. 5. Automated Washing: Aspirate all wells, and then wash plate FIVE times using wash solution. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate

Application Details

	onto absorbent paper or paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes. 6. Add 50 µL Substrate A to each well. 7.Add 50 µL Substrate B to each well. Cover and incubate for 15 minutes at 20-25 °C. (avoid sunlight) 8. Add 50 µL of Stop Solution to each well. Mix well. 9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.
Restrictions:	For Research Use only

Handling

Preservative:	Sodium azide
Precaution of Use:	• This kit contains a small amount of 3, 3', 5, 5'-Tetramethylbenzidine (TMB) in Substrate B. TMB is non-carcinogenic but it is hazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse affected area with plenty of water. • The stop solution provided with this kit is an acid solution. Wear protective gloves, clothing, and face protection. • Care should be taken when handling the standard because of the known and unknown effects of it. • Care should also be taken to avoid contact of skin or eyes with other kit reagents or specimens. In the case of contact, wash immediately with water. • Do not pipette by mouth. • Avoid generation of aerosols. • Waste must be disposed of in accordance with federal, state and local environmental control regulations. • All blood components and biological materials should be handled as potentially hazardous. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour a 121.5°C.
Handling Advice:	• The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance. • Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. • If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary. • Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products. • Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.

Handling

	• Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results. • It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time. • The coefficient of determination of the standard curve should be higher or equal 0.95 and the highest O.D. should be more than 1.0. • Cover or cap all kit components and store at 2-8° C when not in use. • Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity. • Samples should be collected in pyrogen/endotoxin-free tubes. • Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis. • When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis. • When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells. • Do not mix or interchange different reagent lots from various kit lots. • Do not use reagents after the kit expiration date. • Read absorbance immediately after adding the stop solution. • 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. • Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.
Storage:	4 °C
Expiry Date:	6 months

Successfully validated (ELISA (ELISA))

by Celplor LLC

Report Number: 029857

Date: Apr 29 2016

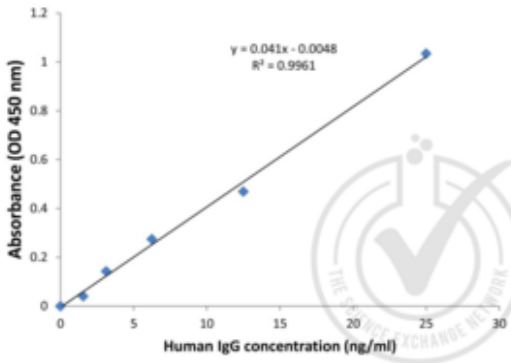
Target:	Human Immunoglobulin G (IgG)
Lot Number:	HU2016ZH021
Method validated:	ELISA (ELISA)
Positive Control:	Normal Human Serum (Sigma, H4522)
Negative Control:	Normal Chicken Serum (Jackson ImmunoResearch, 003-000-001)
Notes:	Human IgG ELISA. Linear range for standard curve is between 0 ng/mL and 25 ng/mL. 1:1000000 dilution of human serum shows the absorbance within the linear range of standard curve.
Controls:	• Positive control: Normal Human Serum (Sigma, H4522) • Negative control: Normal Chicken Serum (Jackson • ImmunoResearch, 003-000-001) • Normal human and chicken serum were diluted with PBS at 1:10, 1:100, 1:1000, 1:10000, • 1:100000 and 1:1000000 • Standard curve: Serial two-fold dilutions of standards (100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0 • ng/mL) were provided in the kit
Protocol:	• All reagents in the ELISA kit were brought up to room temperature (RT) before use. • 1. 100 µL of each sample was added per well to the micro ELISA plate well. All samples and standards were assayed in duplicate. • 2. The plate was covered with sealer (provided in kit) and incubated for 1.5 hour at 37°C. • 3. Liquid was removed from each well by pipette. • 4. Wells were washed with 300 µL wash buffer (1X) five times. Each wash involved fully aspirating the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid. • 5. 100 uL of enzyme conjugate (1X dilution with dilution buffer provided by the kit) was added per well. • 6. The plate was covered with sealer (provided in kit) and incubated for 1.5 hour at 37 °C. • 7. Liquid was removed from each well by pipette. • 8. Wells were washed with 300 µL wash buffer (1X) five times. Each wash involved fully aspirating the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.

- 9. 50 µL of Substrate A and 50 uL of Substrate B were added to each well and the plate was covered with a new plate sealer. The plate was tapped to ensure mixing and incubated for 15 min at 37°C in the dark.
- 10. The reaction was terminated by adding 50 µL of Stop Solution to each well.
- 11. The optical density (OD value) of each well was immediately read using a micro-plate reader set to 450 nm with 570nm as reference.
- 12. A standard curve was generated by plotting the normalized OD value for each standard on the y-axis against the concentration on the x-axis using Excel. A line of best fit through the points on the graph was used to generate the equation X=(Y+0.0048)/0.041.
- 13. The equation X=(Y+0.0048)/0.041 was used to calculate IgG concentrations of the samples based on their normalized average OD values.

Experimental Notes:

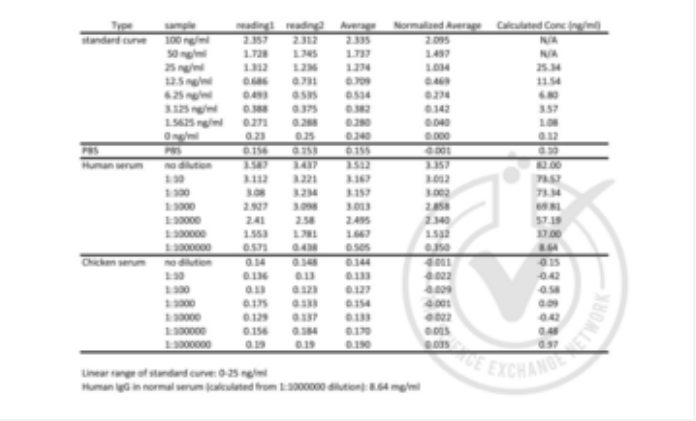
- Only 1:1000000 dilution of human serum has the absorbance within the linear range of standard curve. Therefore at least 1:1000000 dilution is required for the accurate measurement
- of IgG in human serum sample with this kit.
- Weak absorbance was observed in chicken serum at 1:100000 and 1:1000000 dilutions, suggesting weak cross reaction with chicken IgG.

Images for Validation report #029857



Validation image no. 1 for IgG ELISA Kit (ABIN510001)

Figure 1: Graph of corrected-average absorbance (OD 450nm - OD 570nm) readings plotted for standard curve samples. Readings from 50 and 100 ng/mL standard were not included for the generation of standard curve.



Type	sample	reading1	reading2	Average	Normalized Average	Calculated Conc (ng/ml)
standard curve	100 ng/ml	2.357	2.312	2.335	2.095	N/A
	50 ng/ml	1.728	1.745	1.737	1.497	N/A
	25 ng/ml	1.312	1.236	1.274	1.034	25.34
	12.5 ng/ml	0.686	0.731	0.709	0.469	11.54
	6.25 ng/ml	0.493	0.535	0.514	0.274	6.80
	3.125 ng/ml	0.388	0.375	0.382	0.142	3.57
	1.5625 ng/ml	0.271	0.288	0.280	0.040	1.08
	0 ng/ml	0.23	0.25	0.240	0.000	0.12
PBS	PBS	0.156	0.153	0.155	-0.001	0.10
Human serum	no dilution	3.587	3.437	3.512	3.357	82.00
	1:10	3.112	3.221	3.167	3.012	73.57
	1:100	3.08	3.234	3.157	3.002	73.34
	1:1000	2.927	3.098	3.013	2.858	69.81
	1:10000	2.41	2.58	2.495	2.340	57.19
	1:100000	1.553	1.781	1.667	1.512	37.00
	1:1000000	0.571	0.438	0.505	0.350	8.64
Chicken serum	no dilution	0.14	0.148	0.144	-0.011	-0.15
	1:10	0.136	0.13	0.133	-0.022	-0.42
	1:100	0.13	0.123	0.127	-0.029	-0.58
	1:1000	0.175	0.133	0.154	-0.001	0.09
	1:10000	0.129	0.137	0.133	-0.022	-0.42
	1:100000	0.156	0.184	0.170	0.015	0.48
	1:1000000	0.19	0.19	0.190	0.035	0.97

Linear range of standard curve: 0-25 ng/ml
Human IgG in normal serum (calculated from 1:1000000 dilution): 8.64 mg/ml

Validation image no. 2 for IgG ELISA Kit (ABIN510001)

Figure 2: Table of absorbance corrected readings (OD 450nm - OD570nm) for standard curve and serum samples. Value for Average Reading was derived from the average of two readings. PBS was used for the dilution of serum samples. The Average Reading for 0 ng/ml Standard was subtracted from Average Readings of other standards and the Average Reading for PBS was subtracted from Average Readings of serum samples to yield normalized Average Absorbance values. An equation X=(Y+0.0048)/0.041 was generated from the standard curve and used to calculate IgG concentrations shown in the Table.