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

QuicKey Human IgA(Immunoglobulin A) ELISA Kit

Catalog No: E-TSEL-H0019

96T/48T/24T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Phone**:** 240-252-7368(USA) 240-252-7376(USA)

Email**:** techsupport@elabscience.com

Website**:** www.elabscience.com

Please refer to specific expiry date from label on the side of box.

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

QuicKey Series
Get more sensitive and precise results with saving at least 1h comparing to traditional ELISA Kits. The new developed technology in house will help to accelerate your science research in a more efficient way.

Intended use
This ELISA kit applies to the in vitro quantitative determination of Human IgA concentrations in serum, plasma, urine, saliva. Please consult technical support for the applicability if other biological fluids need to be tested.

Specification
●Sensitivity: 1.19ng/mL.
●Detection Range: 6.25-400ng/mL
●Specificity: This kit recognizes Human IgA in samples. No significant cross-reactivity or interference between Human IgA and analogues was observed.
●Repeatability: Coefficient of variation is < 10%.

Background
First described in serum in 1953, IgA is the second dominant isotype in the blood circulation following IgG. It can be found in both monomeric and polymeric forms. Circulating IgA is in monomeric form, whereas secretory IgA, in the mucosal secretions of respiratory, intestinal, and genitourinary systems, is dimeric. In humans, there are two subclasses of IgA: IgA1 and IgA2, constant heavy chains of which are encoded by two separate α1 and α2 genes on chromosome 14 [1]. The main structural difference between them is that IgA2 has a shorter hinge region which may render this isotype more resistant to bacterial proteases in the lumen of gastrointestinal or respiratory systems. In the blood, IgA interacts with an Fc receptor called FcαRⅠ(or CD89), which is expressed on immune effector cells, to initiate inflammatory reactions[2]. Ligation of FcαRⅠ by IgA containing immune complexes causes antibody-dependent cell-mediated cytotoxicity (ADCC), degranulation of eosinophils and basophils, phagocytosis by monocytes, macrophages, and neutrophils, and triggering of respiratory burst activity by polymorphonuclear leukocytes. IgA nephropathy is caused by IgA deposits in the kidneys, it is not yet known why IgA deposits occur in this chronic disease. Some theories suggest an abnormality of the immune system results in these deposits.

1. Kerr M A. Function of immunoglobulin A in immunity[J]. Gut, 2000, 47(6): 751-752.
2. Monteiro R C, Van De Winkel J G J. IgA Fc receptors[J]. Annual review of immunology, 2003, 21(1): 177-204.

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IgA. Samples (or Standards) and biotinylated detection antibody specific for Human IgA are added to the micro ELISA plate wells. Human IgA would combine with the specific antibody. Then Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IgA, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Human IgA. You can calculate the concentration of Human IgA in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 2-8℃ for six months. After test, the unused wells and reagents should be stored according to the table below.

Item	Specifications	Storage conditions after test
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	2-8℃, 1 month
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	Discard unused reconstituted standard and dilutions
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8℃
Biotinylated Detection Ab Working Solution	1 vial, 6 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 μL 48T: 1 vial, 60 μL 24T: 1 vial, 60 μL	2-8℃(Protect from light)
Substrate Reagent	1 vial, 10 mL	
Stop Solution	1 vial, 10 mL	2-8℃
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water

Absorbent paper
Loading slot for Wash Buffer

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch provided in the kit, store it according to the conditions suggested in the above table.
3. Do not reuse the reconstituted standard and HRP conjugate working solution. The unspent biotinylated detection Ab working solution and other stock solutions should be stored according to the storage conditions suggested in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±10 nm. The optical density should be within 0~3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5. Do not mix or use components with those from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

(More detailed information please view our website: http://www.elabscience.com/List-detail-253.html)

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 15 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

Urine: Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at 1000×g at 2~8 ℃.Collect the supernatant to carry out the assay.

Saliva: Remove particulates by centrifugation for 10 minutes at 4000×g at 2-8 ℃. Collect the supernatant to carry out the assay. Recommend to use fresh saliva samples.

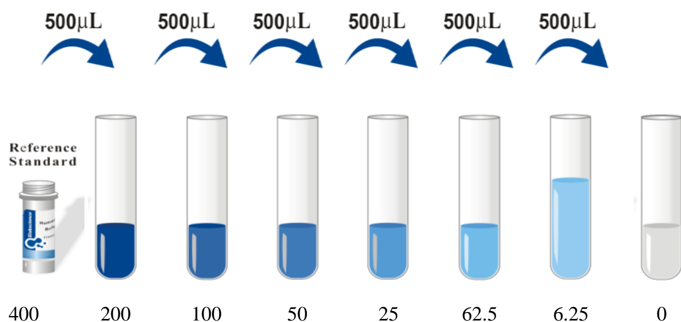
Note for sample

1. Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.
2. Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. Bring samples to room temperature and mix gently.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
4. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
5. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
6. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water to prepare 750mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 400ng/mL(or add 1.0mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 400、200、100、50、25、62.5、6.25、0ng/mL.

Dilution method: Take 7 EP tubes, add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 400ng/mL working solution to the first tube and mix up to produce a 200ng/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



4. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent..

Assay procedure (A brief assay procedure is on the 12th page)

1. Determine wells for **diluted standard**, **blank** and **sample**. Add 50μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Immediately add 50μL of **Biotinylated Detection Ab working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Aspirate or decant the solution from each well, add 350μL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use

immediately after the wash step. Do not allow wells to be dry.

3. Add 100μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37 ℃.
4. Aspirate or decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
5. Add 90μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37 ℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min. Preheat the Microplate Reader for about 15 min before OD measurement.
6. Add 50μLof **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

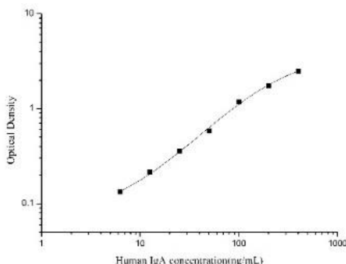
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(ng/mL)	400	200	100	50	25	62.5	6.25	0
OD	2.551	1.807	1.23	0.643	0.421	0.274	0.194	0.061
Corrected OD	2.49	1.746	1.169	0.582	0.36	0.213	0.133	-



Sample values

Serum/Plasma/Urine/Saliva–Samples from apparently healthy volunteers were evaluated for the presence of Human IgA in this assay.

Sample Type	Source	Range	Dilution Factor
Serum（n=12）	Healthy human	0.64-1.48mg/mL	2×10^4-8×10^4
EDTA plasma（n=12）	Healthy human	0.42-1.45mg/mL	1×10^4-8×10^4
Urine（n=12）	Healthy human	135-365ng/mL	2-16
Saliva(n=12)	Healthy human	0.05-0.49mg/mL	500-8000

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Human IgA were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Human IgA were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	19.01	43.71	167.34	17.47	48.35	176.7
Standard deviation	1.09	2.12	8.32	0.88	2.40	9.33
CV (%)	5.73	4.85	4.97	5.04	4.96	5.28

Recovery

The recovery of Human IgA spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	96-105	100
EDTA plasma (n=8)	98-106	101
Urine（n=8）	93-102	97
Saliva(n=8)	80-113	90

Linearity

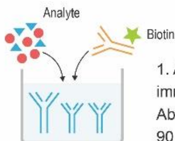
Samples were spiked with high concentrations of Human IgA and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Urine(n=4)	Saliva(n=4)
1:2	Range (%)	88-103	86-97	100-112	88-113
	Average (%)	95	93	104	105
1:4	Range (%)	97-110	94-103	93-102	95-118
	Average (%)	105	99	97	107
1:8	Range (%)	94-109	99-111	85-95	98-119
	Average (%)	101	103	92	111
1:16	Range (%)	93-105	91-104	90-98	88-119
	Average (%)	97	98	93	111

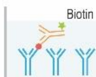
Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

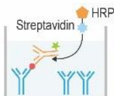
SUMMARY



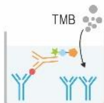
1. Add 50μL standard or sample to the wells, immediately add 50μL Biotinylated Detection Ab working solution to each well. Incubate for 90 min at 37°C



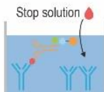
2. Aspirate and wash the plate for 3 times



3. Add 100μL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times



4. Add 90μL Substrate Reagent. Incubate for 15 min at 37°C



5. Add 50μL Stop Solution

6. Read the plate at 450nm immediately. Calculation of the results

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven’t compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.