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Human IgA Antigen ELISA Kit

Catalog # HUIGAKT

Strip well format. Reagents for up to 96 tests.

Rev: October 2017

INTENDED USE

This Human Immunoglobulin A (IgA) antigen assay is intended for the quantitative determination of total human IgA antigen in serum, plasma, hybridoma cell supernatants, ascites or other biological fluids. The assay does not distinguish IgA subclasses. **For research use only.**

BACKGROUND

IgA is the most abundant immunoglobulin in body fluids and the second most abundant immunoglobulin in plasma. IgA in serum is a primarily monomeric 160kDa glycoprotein that initiates defenses against natural infection through interaction with specific receptors and immune mediators. Each monomer consists of two heavy chains and two kappa or lambda light chains. A majority of serum IgA molecules are subclass IgA1 which have longer hinge regions than subclass IgA2.

ASSAY PRINCIPLE

Human IgA will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-human IgA antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human IgA. Color development is directly proportional to the concentration of total IgA in the samples.

STANDARD CALIBRATION

Human IgA standard provided is calibrated against the WHO International Standard for Immunoglobulins G, A and M, Human Serum distributed by NIBSC (67/086), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 617L: 500 ng = 0.040 IU

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human IgA antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human IgA standard:** 1 vial lyophilized standard
- **Anti-human IgA horseradish peroxidase antibody:** 1 vial concentrated polyclonal antibody
- **TMB substrate solution:** 1 bottle of 10ml

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard may be stored at -80°C for later use. Do not freeze-thaw the standard more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 500ng/ml standard solution.

Dilution table for preparation of human IgA standard:

IgA concentration (ng/ml)	Dilutions
100	800µl (BB) + 200µl (from vial)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl IgA standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures IgA antigen in the 0.1-100 ng/ml range. If the unknown is thought to have high IgA levels, dilutions may be made in blocking buffer. A 1:100,000 to 1:800,000 dilution for normal human serum or plasma is suggested for best results.

Antibody Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated antibody into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.4ml of 1:1,000 dilution to 9.6ml of blocking buffer to generate a 1:25,000 dilution. Add 100µl of the 1:25,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

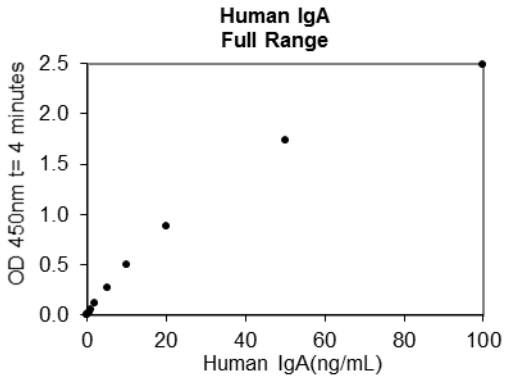
Measurement

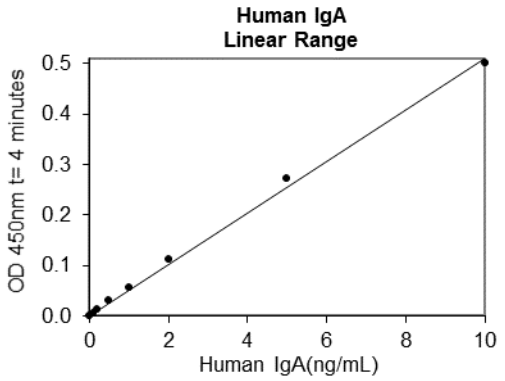
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of IgA in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of IgA in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

The concentration of IgA in normal human serum ranges from 0.7 to 4.0 mg/mL [1].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.044-0.052) and calculating the corresponding concentration. The MDD was 0.083 ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: To assess the linearity of the assay, pooled citrated human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	96	100	101	111
Range	94-99%	98-102%	98-103%	108-114%

Specificity: These studies are currently in progress. Please contact us for more information.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (mg/mL)
Citrate Plasma	1:200,000	2.061
	1:400,000	2.089
	1:800,000	2.015

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Detrick B, Hamilton RG & Folds JD: Manual of Molecular and Clinical Lab Immunology, 7th Edition. 2006.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
B	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
C												
D												
E												
F												
G												
H												