Other ELISA kits are available from ADI (complete list at the web site)

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Monkey: IgM, IgG, IgA, CRP

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF,

Chicken: IgG, IgM, IgY, Ovalbumin

Rabbit: CRP, IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM,

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG

Sheep: IgG

Turkey: IgG

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-1780

Human IgA

ELISA Kit Cat. No. 1780, 96 Tests

For Quantitative Determination of Human IgA in Solution

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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INTENDED USE

The Human IgA ELISA Kit is an in vitro immunoassay for research use in the quantification of human IgA circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of human cells.

INTRODUCTION

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of human immunoglobulins IgG, IgA and IgM, as found circulating in blood or as present in other body fluids, including saliva, milk/colostrum, ascites, tears and mucosa of linings of the gut, respiratory or urogenital tracts.

Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hyper-gammaglobulinemia or acute or chronic infection). Also, measurements of specific antibody levels, in antigen-specific assays, are often best interpreted relative to values of total IgG, IgA, and IgM in the sample and/or individual.

The quantitative immunoassay measures human IgA with high sensitivity; this allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types. Expected performance relative to precision, recovery and linearity of dilution is presented for guidance of use and experimental design.

PRINCIPLE OF THE TEST

The Human IgA ELISA kit is based on the binding of human IgA in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of IgA present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of IgA in samples and control is calculated from a curve of standards containing known concentrations of IgA.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the kit label. Stabilities of the working solutions are indicated under Reagent Preparation.

KIT CONTENTS

Ready For Use: Store as indicated on labels.
To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Human IgA-HRP Conjugate Concentrate (100x) Part No. 1784, 0.15ml	Peroxidase conjugated anti-human IgA antibody in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC storage.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Human IgA-HRP Conjugate Concentrate (100x) Part No. 1784, 0.15ml	Peroxidase conjugated anti-human IgA antibody in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC storage.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent Concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls, and dispose of these samples and containers as biohazard waste.

Standards, Controls, Sample Diluent, and Anti-human IgA-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **other samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , stored refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

QUALITY CONTROL

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgA concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 1:5k are appropriate for most normal human sera. For accuracy, two dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 20ul [1:100] + 980ul diluent = [**1:5k**].

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, to include 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul - 30 min; 5 washes]

- Add 100ul of Working Anti-Human IgA-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

CALCULATIONS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, human IgA concentrations may be determined as follows:

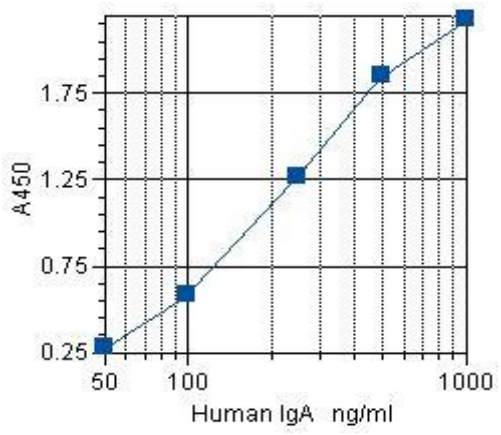
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of human IgA (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The human IgA concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 1000 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	Human IgA ng/ml
1A, B	**Negative Diluent Control**	0.04	0
1C, D	50 ng/ml **Standard**	0.28	50
2E, F	100 ng/ml **Standard**	0.58	100
3G, H	250 ng/ml **Standard**	1.26	250
2A, B	500 ng/ml **Standard**	1.85	500
2C, D	1000 ng/ml **Standard**	2.18	1000
2E, F	**Positive Serum Control** [Value: 112 - 208 ng/ml]	0.79	133
2G, H	**Sample** [Diluted 1:5k]	1.86	508
	Calculated: 5k-fold dilution x 508 ng/ml = **2.54** mg/ml in serum		

A typical assay Standard Curve (do not use for calculating sample values)



PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with IgA, and have essentially no reactivity with IgG, IgM, IgE or any other human serum proteins.

Serum from the following species showed no significant reactivity at 1:500 dilution: hamster, guinea pig, bovine, pig, horse, sheep, goat, dog, cat, rabbit or chicken; also 10% neonatal bovine serum. Monkey showed slight cross-reactivity.

Normal Range

Assay of IgA in stored sera from twelve (12) individual adult humans ranged from 0.3 to 2.3 mg/ml. Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgA were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations (CVs) were calculated for the concentrations using a point-to-point curve-fitting program.

IgA concentrations were measured with very good within-assay (3.6 to 9.8 %CV) and between-assay (5.7 to 7.9 %CV) reproducibility.

Sample	IgA ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	141	4.6	5.7
Mid Sample	358	3.6	7.9
High Sample	521	9.8	6.1

Linearity of Dilution

Three (3) individual stored sera and purified human IgA were diluted to 2 levels for testing, and concordance of the assay values was compared. Agreement of values ranged from 94 to 99%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Serum 1	1:2k 1:16k	806 95.6	1.61 1.53	**97 %**
Serum 2	1:2.5k 1:20k	860 106	2.15 2.11	**99 %**
Serum 3	1:2.5k 1:20k	613 68.3	1.53 1.37	**94 %**
Purified IgA	1:1k 1:8k	564 69	0.56 0.55	**99 %**

PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery
High and low concentrations of human IgA were mixed into each of 3 serum samples. Observed assay values compared to expected values ranged from 102 to 117%, indicating accurate quantification of IgA in human serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High IgA Concn		357	
+ Human serum A, 225 ng/ml	582	680	**117**
+ Human serum B, 174 ng/ml	531	542	**102**
+ Human serum C, 177 ng/ml	534	604	**113**
Low IgA Concn		119	
+ Human serum A, 225 ng/ml	344	350	**102**
+ Human serum B, 174 ng/ml	293	306	**104**
+ Human serum C, 177 ng/ml	296	312	**105**

ELISA Kit Components	Amount	Part No.
Anti-Human IgA Microwell Strip Plate	8-well strips (12)	1781
Human IgA Control	0.65 ml	1782
Human IgA Standard 50 ng/ml	0.65 ml	1783B
Human IgA Standard 100 ng/ml	0.65 ml	1783C
Human IgA Standard 250 ng/ml	0.65 ml	1783D
Human IgA Standard 500 ng/ml	0.65 ml	1783E
Human IgA Standard 1000 ng/ml	0.65 ml	1783F
Anti-Human IgA HRP Conjugate (100X)	0.15 ml	1784
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-1780

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

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