IgA (Human) ELISA Kit

Catalog Number KA2110

96 assays

Version: 05

Intended for research use only

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Introduction

Background

Human Immunoglobulin A (IgA) is the most abundant antibody isotype in mucosal secretions and exists in two subclasses IgA1 and IgA2 (1). While circulating serum IgA1 occurs mainly in the monomeric 160 kDa form (2), mucosal secretary IgA2 is in dimeric form and serves as the first line of defense against microorganisms through immune exclusion (3). Selective IgA deficiency is the most common primary immunodeficiency observed by a maturation defect in B cells to produce IgA (4). IgA nephropathy is the primary glomerulonephritis characterized by IgA deposition in the kidney and associated with a dysregulation of the immune response (5-6).

Principle of the Assay

The IgA (Human) ELISA Kit is designed for detection of human IgA in plasma, serum, urine, saliva, milk, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human IgA in less than 4 hours. A polyclonal antibody specific for human IgA has been pre-coated onto a 96-well microplate with removable strips. IgA in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for IgA, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Human IgA Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human IgA.	96 (8x12) wells
Sealing Tapes: Pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human IgA Standard: Human IgA in a buffered protein base, lyophilized	200 ng
Biotinylated Human IgA Antibody (60x): A 60-fold concentrated biotinylated polyclonal antibody against IgA.	120 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate.	80 µL
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and reseal. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution.

Assay Protocol

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- Standard Curve: Reconstitute the 200 ng (24 mU/mL) of Human IgA Standard with 2 mL of MIX Diluent to generate a 100 ng/mL (12 mU/mL) standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (100 ng/mL) 1:2 with MIX Diluent to produce 50, 25, 12.5, 6.25, 3.125, 1.563 0.781 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and use within 30 days.

Standard Point	Dilution	[IgA] (ng/mL)	[IgA] (mU/mL)
P1	1 part Standard (100 ng/mL) + 1 part Mix Diluent	50.00	6.000
P2	1 part P1 + 1 part MIX Diluent	25.00	3.000
P3	1 part P2 + 1 part MIX Diluent	12.50	1.500
P4	1 part P3 + 1 part MIX Diluent	6.250	0.750
P5	1 part P4 + 1 part MIX Diluent	3.125	0.375
P6	1 part P5 + 1 part MIX Diluent	1.563	0.188
P7	1 part P6 + 1 part MIX Diluent	0.781	0.094
P8	MIX Diluent	0.000	0.000

- Biotinylated Human IgA Antibody (60x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:60 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Sample Preparation

- Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:80000 into MIX Diluent or within the range of 1:20000 to 1:200000, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. Dilute samples 1:80000 into MIX Diluent or within

the range of 1:20000 to 1:200000, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute Urine 1:20 with MIX Diluent or within the range of 1:10 to 1:100, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute Saliva 1:2000 with MIX Diluent or within the range of 1:1000 to 1:10000, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute Milk 1:10000 with MIX Diluent or within the range of 1:2000 to 1:40000, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:500 into MIX Diluent or within the range of 1:200 to 1:2000, and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Guidelines for Dilutions of 1:100 or Greater (for reference only; please follow the protocol for specific dilution suggested)	
1:100	1:10000
A) 4 µL sample: 396 µL buffer (100x) = 100 fold dilution *Assuming the needed volume is less than or equal to 400 µL.*	A) 4 µL sample: 396 µL buffer (100x) B) 4 µL of A: 396 µL buffer (100x) = 10000 fold dilution *Assuming the needed volume is less than or equal to 400 µL.*
1:1000	1:100000
A) 4 µL sample: 396 µL buffer (100x) B) 24 µL of A: 216 µL buffer (10x) = 1000 fold dilution *Assuming the needed volume is less than or equal to 240 µL.*	A) 4 µL sample: 396 µL buffer (100x) B) 4 µL of A: 396 µL buffer (100x) C) 24 µL of B: 216 µL buffer (10x) = 100000 fold dilution *Assuming the needed volume is less than or equal to 240 µL.*

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

3. Add 50 µL of Human IgA standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents; hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of Biotinylated Human IgA Antibody to each well and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

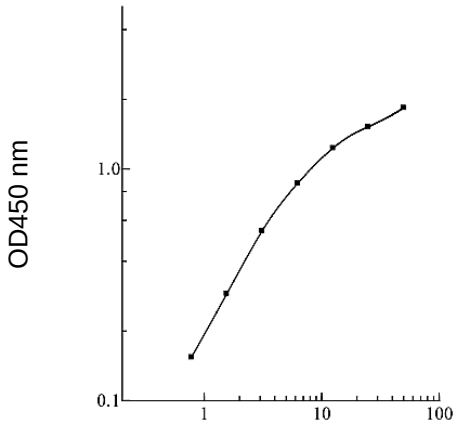
Data Analysis

Calculation of Results

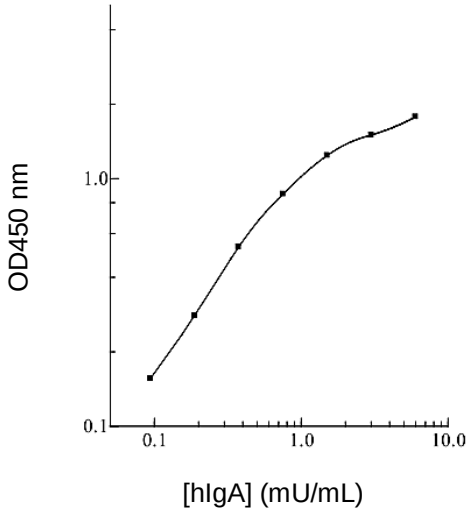
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

- ✓ The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Human IgA Standard Curve



Human IgA Standard Curve



Performance Characteristics

- ✓ The minimum detectable dose of IgA is typically ~ 0.7 ng/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 5.0% and 7.2% respectively.
- ✓ Kit standard has been calibrated against WHO international standard.

- Linearity

Sample Dilution	Plasma	Serum
1:40000	92%	91%
1:80000	99%	100%
1:160000	105%	103%

Sample Dilution	Saliva
1:1000	94%
1:2000	101%
1:4000	106%

Sample Dilution	Urine
1:10	89%
1:20	96%
1:40	104%

- Recovery

Standard Added Value	3.13 - 25 ng/mL
Recovery %	85 - 113 %
Average Recovery %	98%

- Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	< 5%
Mouse	None
Rat	None
Swine	None
Rabbit	None

Immunoglobulins	% Cross Reactivity
IgM	< 5%
IgA1	100%
IgA2	100%
IgG1	< 1%
IgG2	None
IgG3	None
IgG4	None
IgD	< 1%
IgE	< 1%

Resources

References

1. Delacroix DL *et al.* (1982) *Immunology* 47:383-385
2. Kerr MA (1990) *Biochem. J*. 271:285-296
3. Corthésy B (2007) *J Immunol.* 178(1):27-32
4. Yel L (2010) *J Clin Immunol.* 30(1):10–16
5. D'Amico G (1987) *Quarterly J. Med*. 64(245):709–727
6. Pettersson E (1997) *J Intern Med.* 242(5):349-353

Plate Layout

12								
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	A	B	C	D	E	F	G	H