REFERENCES

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Human IgA Immunoassay

Catalog Number: SEKH-0207

For the quantitative determination of human IgA concentrations in cellculture supernate, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of IgA in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	104	108
	Range (%)	93—114	98—117
1:4	Average% of Expected	105	109
	Range (%)	96—113	99—118
1:8	Average% of Expected	103	104
	Range (%)	96—109	93—114
1:16	Average% of Expected	102	103
	Range (%)	93—107	97—108

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 40pg/mL.

Specificity The following recombinant human proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference. BMP1, BMP2, BMP4, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, TGFβ 1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, VEGF.IFN

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of IgA spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IgA in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	98	89—95
Cell culture supernatants	103	94—111

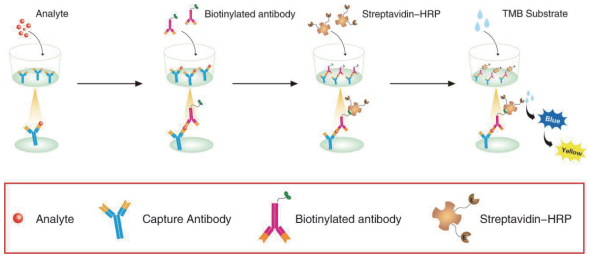
BACKGROUND

Immunoglobulin A (IgA) is an antibody that plays a critical role in mucosal immunity. More IgA is produced in mucosal linings than all other types of antibody combined; between three and five grams are secreted into the intestinal lumen each day. This accumulates to 75% of the total immuno-globulin produced in the entire body. IgA has two subclasses (IgA1 and IgA2) and can exist in a dimeric form called secretory IgA (sIgA). In its secretory form, IgA is the main immunoglobulin found in mucous secretions, including tears, saliva, colostrum and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium. It is also found in small amounts in blood. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, thus sIgA can survive in the harsh gastrointestinal tract environment and provide protection against microbes that multiply in body secretions.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IgA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgA present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IgAis added to detect the captured IgA protein in sample. For signal development, horseradish peroxidase (HRP)-conjugatedStreptavidinis added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



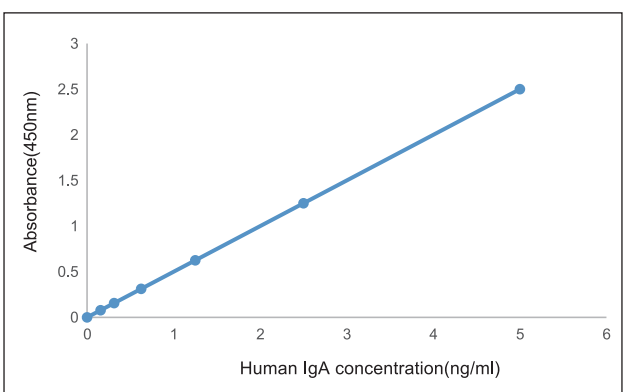
TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the IgA ELISA

Standard(ng/ml)	O.D.1	O.D.2	Average	Corrected
0	0.038	0.043	0.041	-
0.08	0.150	0.145	0.147	0.107
0.2	0.218	0.210	0.214	0.174
0	0.324	0.313	0.319	0.278
0.625	0.515	0.497	0.506	0.466
1.25	0.837	0.807	0.822	0.781
2.5	1.362	1.314	1.338	1.298
5	2.220	2.141	2.181	2.140



Representative standard curve for IgA ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100µl standard or samples to each well, incubate 120 minutes, at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human IgA antibody to each well, incubate 60 minutesat room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-30 minutes, at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the IgAconcentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,20 ng/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
ConcentratedBiotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
ConcentratedStreptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent- 16ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.
6.HumanIL-1 beta controls (optional; available from Solarbio).

SPECIMEN COLLECTION & STORAGE

Cell Culture supernate- Centrifuge cell culture media at 1000×gto remove debris. Assay immediately oraliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at roomtemperature or overnight at 2-8℃.Centrifuge at approximately for 15 minutes at 1000×g. Assayimmediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and storesamples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

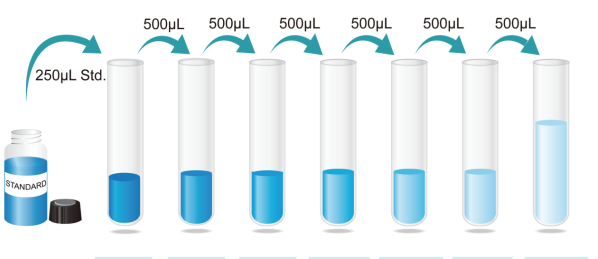
REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentratewith 570mL of deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mixgently until the crystals have completely dissolved.
3. **Standard\Sample**- Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 20 ng/mL. Allow the standard to sit for a minimum of 15 minutes with

gentle agitation prior to making dilutions. Pipette 750μL of Standard/Sample Diluent into the 5 ng/mL tube, and add 250μLstock solution of 20 ng/mL into it to get the high standard of 5 ng/mL. Pipette 500μL of Standard/Sample Diluent into the remaining tubes. Use the high standard to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 5 ng/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 ng/mL).

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-humanIgAantibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**



Preparation of IgA standard dilutions