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Mouse IgA Immunoassay

Catalog Number: SEKM-0094

For the quantitative determination of mouse IgA concentrations in cell culture supernates, 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)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	97	106
	Range (%)	91—103	99—112
1:4	Average% of Expected	102	107
	Range (%)	95—108	101—113
1:8	Average% of Expected	99	101
	Range (%)	86—103	90—117
1:16	Average% of Expected	96	103
	Range (%)	92—105	95—115

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 390pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant mouse IgA. The factors listed below were prepared at 16ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant mouse	Natural proteins	Recombinant human
IgM		
IgG		

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.

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	96	86－106
Cell culture supernatants	104	96－112

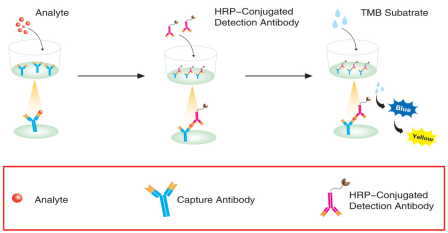
BACKGROUND

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 immunoglobulin 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. IgA is a poor activator of the complement system, and opsonisesonly weakly. Its heavy chains are of the type α. In the blood, IgA interacts with an Fc receptor called FcαRI (or CD89), which is expressed on immune effector cells, to initiate inflammatory reactions.

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 HRP-conjugated antibody specific for IgA is added to detect the captured IgA protein in sample. The wells are then washed to remove unbound HRP-conjugated antibody and Tetramethyl-benzidine (TMB) reagent is added. Incubated at room temperature, only those wells that contain IgA, HRP-conjugated antibody will appear blue in color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450nm. The OD value is proportional to the concentration of Mouse IgA. You can calculate the concentration of Mouse IgA in the sample by comparing the OD of the sample to the standard cure.

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.

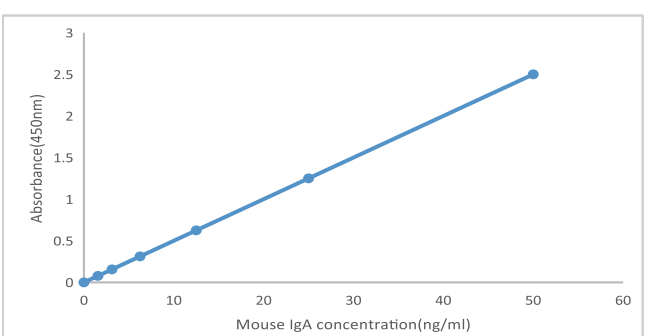
PRECAUTIONS

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.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration 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

Standared(ng/ml)	OD.	OD.	Average	Corrected
0	0.048	0.057	0.052	-
0.78	0.172	0.173	0.173	0.120
1.6	0.250	0.252	0.251	0.199
3	0.372	0.375	0.373	0.321
6.25	0.591	0.595	0.593	0.541
12.5	0.960	0.967	0.963	0.911
25	1.563	1.574	1.568	1.516
50	2.546	2.564	2.555	2.503



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, shock incubate 120 minutes room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µL working solution of HRP-Conjugate anti-Mouse IgA to each well, seal the plate and incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

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

⇩

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

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 IgA concentrations versus the log of the O.D. and the best fit line can be determined by

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,50ng/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
HRP Congugated Antibody (100×) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	4 bottles	Store at 2-8°C **for six months
HRP Congugated Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
20 × Wash Buffer Concentrate- 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.

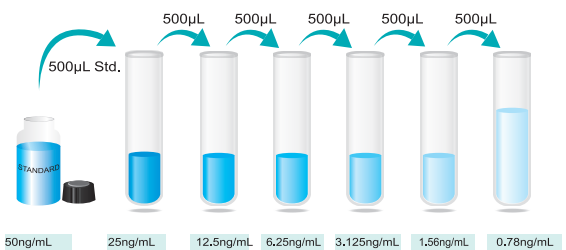
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot 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 room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately 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 for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

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 Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Sample** - Reconstitute the Standard with 1mL of ultrapure.This reconstitution produces a stock solution of 50ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500uL of Standard/Sample Diluent into 25ng/ml tube and the remaining tubes. Use the stock solution of 50ng/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 50ng/mL standard serves as the high standard. The Standard /sample Diluent as the zero standard (0ng/mL).



Preparation of IgA standard dilutions

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

4. **Working solution of HRP-Congugated Antibody(100×)**- Centrifuge for 1 min at 6000 × g to bring down the material prior to open the vial. The vial contains 120μL HRP Conjugate sufficient for a 96-well plate. Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100μL of HRP Conjugate to 10mL of **HRP-Congugated Diluent** to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE.
***The working solution should be used within one day after dilution.**