Sheep IgA Immunoassay

Catalog Number: SEKS-0001

For the quantitative determination of Sheep IgA concentrations in cell culture supernates, serum, and plasma.

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

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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 opsonises only weakly. Its heavy chains are of the type α . In the blood, IgA interacts with an Fc receptor called Fcα RI (or CD89), which is expressed on immune effector cells, to initiate inflammatory reactions. Ligation of Fc α RI by IgA containing immune complexes causes antibodydependent cell-mediated cytotoxicity (ADCC), degranulation of eosinophils and basophils, phagocytosis by monocytes, macrophages, and neutrophils, and triggering of respiratory burst activity by polymorphonuclear leukocytes. Polymeric IgA (mainly the secretory dimer) is produced by plasma cells in the lamina propria adjacent to mucosal surfaces. It binds to the polymeric immunoglobulin receptor on the basolateral surface of epithelial cells, and is taken up into the cell via endocytosis. The receptor-IgA complex passes through the cellular compartments before being secreted on the luminal surface of the epithelial cells, still attached to the receptor. Proteolysis of the receptor occurs, and the dimeric IgA molecule, along with a portion of the receptor known as the secretory component, are free to diffuse throughout the lumen. In the gut, it can bind to the mucus layer on top of the epithelial cells to form a barrier capable of neutralizing threats before they reach the cells. Decreased or absent IgA, termed selective IgA deficiency, can be a clinically significant immunodeficiency. Neisseria gonorrhœ ae (which causes gonorrhea), Streptococcus pneumoniae, and Haemophilus influenzae type B all releases a protease which destroys IgA.

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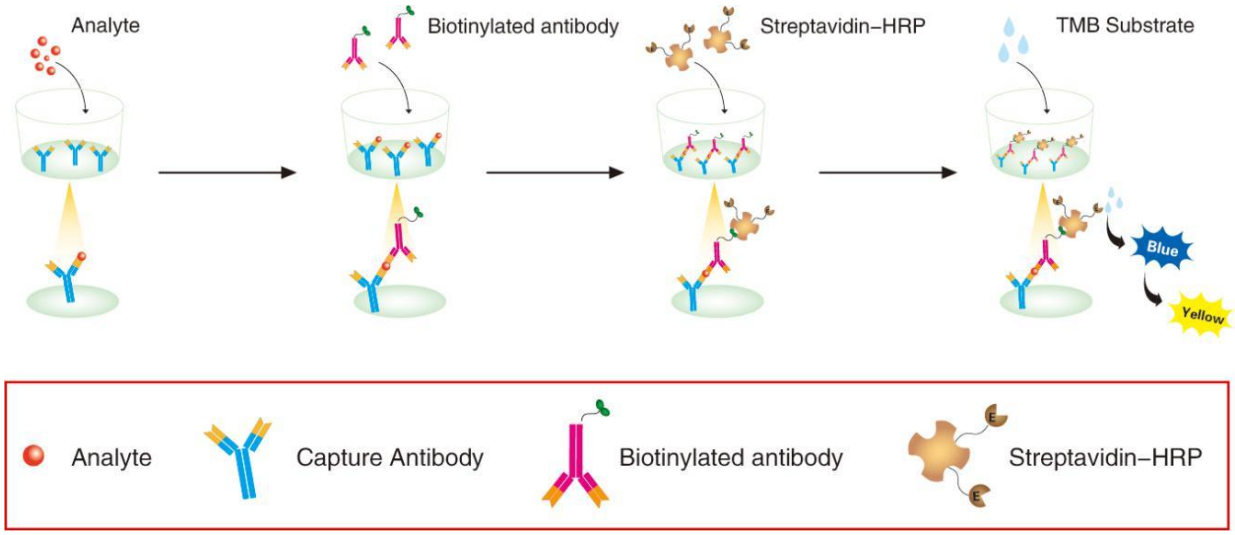
PRINCIPLE OF THE ASSAY

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 IgA is added to detect the captured IgA

protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is 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.

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,7000pg/ml upon reconstitution	2 vials	Aliquot and Store at -20℃** for six months
lyophilized Biotin-Conjugated antibody	1 vials	Store at 2-8℃ **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8℃** for six months
Standard /sample Diluent	1 bottle	Store at 2-8℃** for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8℃** for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8℃** for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8℃** for six months
Substrate Solution	1 bottle	Store at 2-8℃** for six months
Stop Solution	1 bottle	Store at 2-8℃** 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

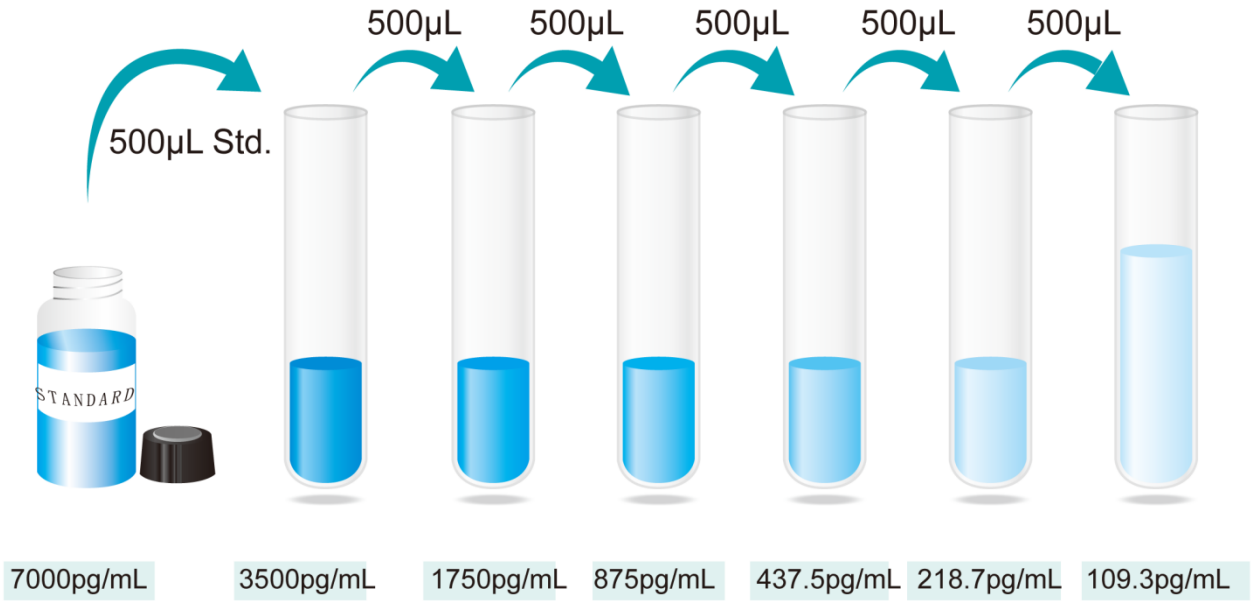
Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 ℃. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 ℃. 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 ℃. Avoid repeated freeze-thaw cycles.

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 20x 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 (2 vials)** - Sheep IgA Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of **Standard/Sample Diluent**. This reconstitution produces a stock solution of 7000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.Pipette 500μL of Standard/Sample Diluent into 3500pg/ml tube and the remaining tubes. Use the stock solution of 7000pg/mL to produce a 2-fold dilution series (below). Mix each

tube thoroughly(vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 7000 pg/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0 pg/mL).



Preparation of Sheep 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 Biotin-Conjugate anti-Sheep IgA antibody(1 vials)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add **110 μL** of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take **50μL** of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used. make a **1:200** 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(120μL)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 μL HRP Conjugate sufficient for a 96-well plate.Make **1:100** dilutions in Reagent Diluent. If the entire 96-well plate is used, add **100 ul** of HRP Conjugate to 10 mL of Streptavidin-HRP 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.**

ASSAY PROCEDURE

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

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Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-Sheep IgA antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

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

CALCULATION OF RESULTS

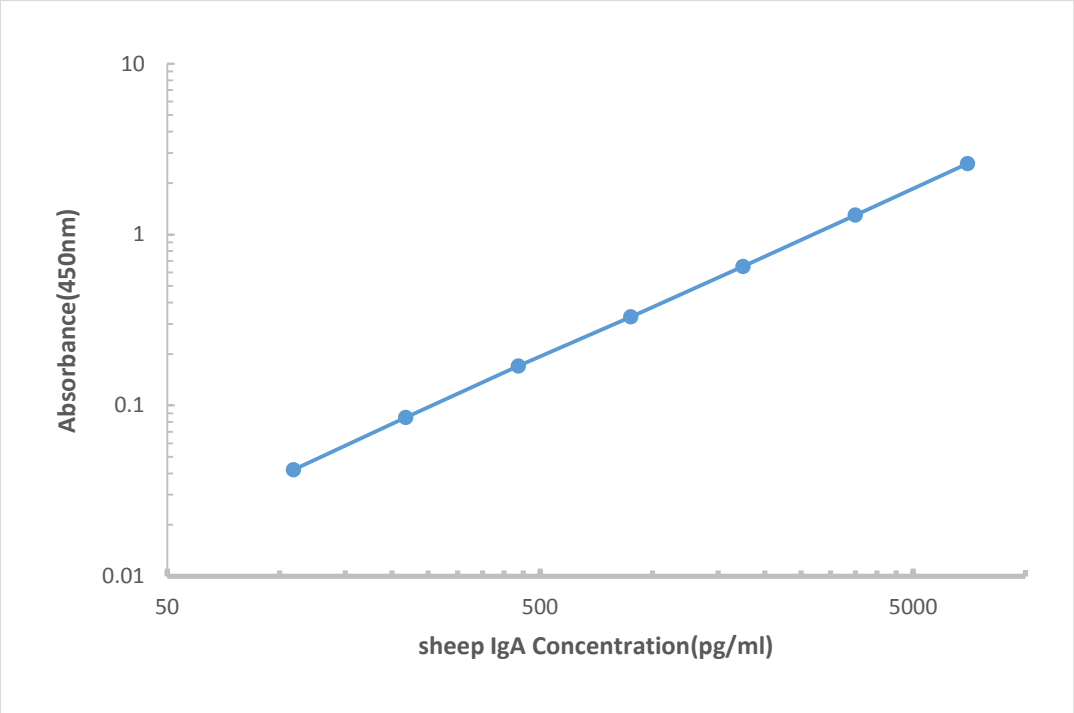
1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using 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 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

Std (pg/mL)	O.D.1	O.D.2	Averag	Correct
0	0. 047	0. 05	0. 048	---
109.375	0. 185	0. 187	0. 186	0. 137
218.75	0. 275	0. 263	0. 269	0. 220
437.5	0. 565	0. 581	0. 573	0. 524
875	0. 875	0. 851	0. 863	0. 814
1750	1. 218	1. 235	1. 226	1. 178
3500	1. 896	1. 873	1. 884	1. 836
7000	2. 574	2. 554	2. 564	2. 515



Representative standard curve for IgA ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 27 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Sheep IgA. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

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, IFNγ, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, VEGF

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	94	85-103
Cell culture supernatants	93	87-105

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	95	103
	Range (%)	86-104	95-116
1:4	Average% of Expected	97	106
	Range (%)	89-107	98-115

REFERENCES

1. S Fagarasan and T Honjo (2003). Nat. Rev. Immunology 3 (1): 63–72.
2. P. Brandtzaeg, R. Pabst (2004). Trends Immunology 25 (11): 570–577.
3. AJ Macpherson and E Slack. (2007). Curr Opin Gastroenterol. 23 (6): 673–678.
4. CS Kaetzel et al. (1991). Vet. Res. 37 (3): 455–67.
5. CS Kaetzel et al. (1991) Proc Natl Acad Sci USA 88 (19): 8796–8800.