abcam

Product datasheet

Human IgA ELISA Kit ab 196263

Recombinant SimpleStep ELISA

10 References 8 Images

Overview

Product name

Human IgA ELISA Kit

Detection method

Colorimetric

Precision

Sample	n	Mean	SD	CV%
Serum	8			4.12%

Inter-assay

Intra-assay

Sample	n	Mean	SD	CV%
Serum	3			5.92%

Cell culture supernatant, Saliva, Milk, Urine, Serum, Plasma Sample type

Assay type Sandwich (quantitative)

Sensitivity 0.25 ng/ml

Range 0.78 ng/ml - 50 ng/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Saliva	90.31	87.91% - 92.45%
Milk	92.21	81% - 101.51%
Urine	99.48	86.21% - 109.34%
Serum	98.37	81.35% - 109.17%
Cell culture media	100.65	85.17% - 117.29%
Hep Plasma	97.17	91.05% - 109.18%

Sample type	Average %	Range
EDTA Plasma	81.23	75.01% - 87.76%
Cit plasma	93.47	88.78% - 98.99%

Assay time

1h 30m

Assay duration

One step assay

Species reactivity

Reacts with: Human

Does not react with: Goat, Cow, Pig

Product overview

Human IgA ELISA Kit (ab196263) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of IgA protein in cell culture supernatant, milk, saliva, serum, urine, and plasma. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human IgA with 0.25 ng/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (ab203359) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

ASSAY SPECIFICITY

This kit recognizes both native and purified Human IgA protein in serum, plasma, milk, urine, saliva, and cell culture media samples only.

Cell and tissue extracts have not been tested with this kit.

CROSS REACTIVITY

Human IgG, human IgM, and human IgE were prepared at 20 ng/mL and 10 ng/mL in Sample Diluent NS and assayed for cross reactivity. No significant cross reactivity was observed for human IgG, human IgM, or human IgE at either concentration with a mean OD deviation from background of 0.006.

INTERFERENCE

Purified human IgA was assayed at 20 ng/mL in the presence and absence of 20 ng/mL of human

IgM or human IgE to determine interference. After background subtraction, human IgM and IgE were found to interfere with the assay by 9.24%. Human IgA was assayed at 20 ng/mL in the presence and absence of 80 ng/mL of human IgG to determine interference. After background subtraction, no significant interference was observed.

SPECIES REACTIVITY

This kit recognizes human IgA protein.

Other species reactivity was determined by measuring 1:200,000 (dilution) serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Hamster, Guinea Pig, Rabbit, Dog, Goat, Pig, Cow

CALIBRATION

This immunoassy is calibrated against a highly purified human lgA. The NIBSC/WHO unclassified purified human lgG/lgM/lgG preperation 67/086. was evaluated in this kit.

The dose response curve of the unclassified standard parallels the SimpleStep standard curve. To Convert sample values obtained with the SimpleStep IgA kit to approximate NIBSC International units, use the equation below.

NIBSC 67/086 approximate value (IU/mL) = 0.000064 (6.4e-5 IU) x SimpleStep IgA value (ng/mL)

Human Immunoglobulin A (IgA) belongs to the immunoglobulin family of proteins which include Human IgG, IgE, and IgM, and are known as antibodies. IgA has an essential role in mucosal immunity. IgA is the second most common serum Ig and is about one-fifth of the concentration of IgG, however it is the most abundant immunoglobulin in secretions, such as saliva, mucus, sweat, and tears. In normal human serum, IgA is found mainly as a monomeric, alternatively, in secretions, IgA is found as a dimer containing two additional proteins, secretory piece, and a J chain. The secretory piece is synthesized in the epithelial cells and is added to IgA as it passes into the secretions, protecting it from degradation. IgA works is with Iysozymes to hydrolyze carbohydrates in bacterial cells walls to clear the infection.

The most common disease associated with high levels of IgA in the kidney is IgA nephropathy, aka Berger's disease. There is no clear clarification as to why IgA deposits occur in the kidney, however abnormality in the immune system is a possibility. Other diseases that IgA is associated with are Celiac disease and Henoch-Schonlein purpura (HSP).

Microplate

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Notes

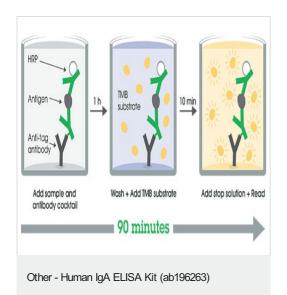
Platform

Components	1 x 96 tests	1 x 384 tests	1 x 96 tests
10X Human lgA Capture Antibody	1 x 600µl	1 x 600µl	1 x 600µl
10X Human IgA Detector Antibody	1 x 600µl	1 x 600µl	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml	2 x 20ml	1 x 20ml
384 well CaptSure™ microplates	0 x 0 unit	1 unit	0 x 0 unit
Antibody Diluent CPI2	1 x 6ml	1 x 6ml	1 x 6ml
Human IgA Lyophilized Purified Protein	2 vials	2 vials	2 vials
Plate Seals	1 unit	1 unit	1 unit
Sample Diluent NS (ab193972)	1 x 50ml	1 x 500ml	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	0 x 0 unit	1 unit
Stop Solution	1 x 12ml	2 x 12ml	1 x 12ml
TMB Development Solution	1 x 12ml	2 x 12ml	1 x 12ml

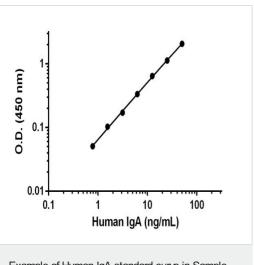
Relevance

Human IgA (immunoglobulin A) is a glycosylated protein of 160 kDa and is produced as a monomer or as a J chain linked dimer. Monomeric IgA constitutes 5-15 % of the serum immunoglobulins whereas dimeric IgA is localized to mucosa surfaces such as saliva, gastrointestinal secretion, bronchial fluids and milk. Mucosal IgA plays a major role in host defence by neutralising infectious agents at mucosal surfaces. The production is usually local and antigen specific IgA producing B cells can be found in regions under the lamina propria where they mature into dimeric IgA producing plasma cells. IgA deficiency is the most common immunodeficiency that may affect both serum and mucosal produced IgA. OR: The secretory component is a component of immunoglobulin A (IgA) which consists of a portion of the polymeric immunoglobulin receptor. Polymeric IgA binds to the polymeric immunoglobulin receptor on the basolateral surface of epithelial cells and is taken up into the cell via transcytosis. 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 the secretory component, are free to diffuse throughout the lumen.

Images



SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



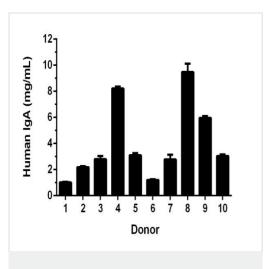
Example of Human IgA standard curve in Sample Diluent NS.

Background-subtracted data values (mean +/- SD) are graphed.

Conc.	O.D. 4	150 nm	Mean
(ng/mL)	1	2	O.D.
0	0.065	0.075	0.069
0.78	0.121	0.123	0.122
1.56	0.175	0.174	0.174
3.13	0.239	0.247	0.243
6.25	0.410	0.403	0.407
12.5	0.716	0.716	0.716
25	1.269	1.132	1.200
50	2.119	2.163	2.141

Example of Human IgA standard curve in Sample Diluent NS. The Human IgA standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

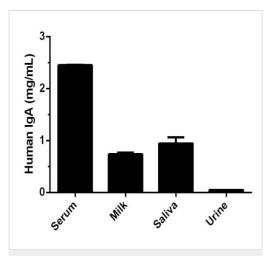
Standard curve



lgA in serum using this assay. Results were interpolated from the standard curve in Sample Diluent NS and corrected for sample dilution (1:2X10 5). The mean level of Human lgA was found at 3.978 mg/mL with a range of 1.016 – 9.476 mg/mL.

Ten individual healthy donors were evaluated for the presence of

IgA levels in individual healthy donors.



Comparison of IgA levels in Human serum, milk, saliva, and urine.

Fluids from a pooled selection of healthy donors were evaluated for the presence of Human IgA in serum, milk, saliva, and urine using this assay. Results were interpolated from the standard curve in Sample Diluent NS and corrected for sample dilution (1:2X10 5). The mean levels of Human IgA in serum were found at 2.45 mg/mL, in milk at 0.74 mg/mL, in saliva at 0.94 mg/mL and in urine at 0.05 mg/mL.

Dilution Factor	Interpolated value	1:50,000 Human Serum	1:25,000 Human Plasma (Citrate)	Human Plasma	1:50,000 Human Plasma (Heparin)	1:10 Culture Media (RPMI
Undiluted	ng/mL	39.45	65.20	62.91	44.01	7.15
Unallutea	% Expected value	100	100	100	100	100
2	ng/mL	18.79	34.25	33.26	22.57	3.89
	% Expected value	95	107	106	103	109
4	ng/mL	9.12	16	15.70	11	1.91
	% Expected value	92	100	100	100	107
8	ng/mL	4.42	7.86	7.86	5.27	0.98
	% Expected value	90	98	100	96	110
	ng/mL	2.16	3.96	3.93	2.67	0.4
16	% Expected value	88	99	100	97	100

Linearity of dilution.

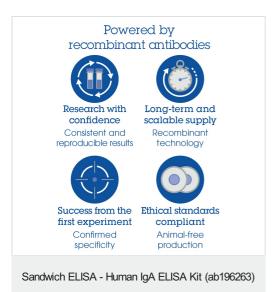
Native IgA wa	as measured in serum, plasma citrate, plasma EDTA,
plasma hepa	rin, milk, saliva and urine in a 2-fold dilution series.
Sample diluti	ons were made in Sample Diluent NS. Purified lgA
was spiked in	n culture media and diluted in a 2-fold dilution series in
Sample Dilue	ent NS.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Dilution Factor	Interpolated value	1:10,000 Human Milk	1:13,000 Human Saliva	1:32 Human Urine
l landilista d	ng/mL	56.744	64.623	54.42
Undiluted	% Expected value	100	100	100
2	ng/mL	28.90	29.77	25.65
	% Expected value	102	92	94
4	ng/mL	14.30	15.88	13.24
	% Expected value	101	98	97
8	ng/mL	7.22	8.36	6.98
	% Expected value	102	104	103
16	ng/mL	3.68	4.71	3.69
	% Expected value	104	107	109

Linearity of dilution.

Purified IgA was spiked in culture media and diluted in a 2-fold dilution series in Sample Diluent NS.



To learn more about the advantages of recombinant antibodies see **here**.

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