abcam

Product datasheet

Human IgG ELISA Kit ab195215

SimpleStep ELISA

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Overview

Product name

Human IgG ELISA Kit

Detection method

Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Serum	8			6.4%

Inter-assay

Sample	n	Mean	SD	CV%
Serum	3			14.7%

Sample type

Cell culture supernatant, Saliva, Milk, Urine, Serum, Hep Plasma, EDTA Plasma, Cit plasma,

Tissue Lysate, Cerebral Spinal Fluid

Assay type

Sandwich (quantitative)

Sensitivity

0.02 ng/ml

Range

0.23 ng/ml - 15 ng/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Saliva	89	80% - 106%
Milk	89	83% - 94%
Urine	87	82% - 93%
Serum	101	88% - 125%
Cell culture media	107	96% - 115%
Hep Plasma	100	100% - 100%

1

Sample type	Average %	Range
EDTA Plasma	90.17	87% - 93%
Cit plasma	100	98% - 102%
Cerebral Spinal Fluid	98	88% - 105%

Assay time

1h 30m

Assay duration

One step assay

Species reactivity

Reacts with: Human

Does not react with: Sheep, Goat, Cow

Product overview

Human IgG ELISA kit (ab195215) is designed for the quantitative measurement of IgG protein in human serum, plasma, milk, urine, saliva, culture media and tissue extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human IgG with 20 pg/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 SimpeStep ELISA® kits.

CROSS REACTIVITY

Human IgM, human IgA and human IgE were prepared at 10 ng/mL and 250 ng/mL in Sample Diluent NS and assayed for cross reactivity. No cross-reactivity was observed for IgM or IgE at either concentration with a mean OD deviation from background of -0.01. No cross-reactivity was observed for IgA at 10 ng/mL and only 0.4% cross-reactivity at 250 ng/mL of IgA.

INTERFERENCE

Purified human IgG was assayed at 5 ng/mL in the presence and absence of 250 ng/mL of human lgM, human lgA and human lgE to determine interference. After background subtraction, recovery of human IgG was observed at a mean of 98% with a standard deviation of 0.06.

SPECIES REACTIVITY

This kit recognizes human IgG protein.

Other species reactivity was determined by measuring 10 ng/mL of purified IgG from various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration of human IgG assayed at the same concentration.

Reactive species: Rhesus Monkey

Reactivity < 3% was determined for the following species: Mouse, Rat, Rabbit, Dog, Goat, Sheep,

Cow, Guinea Pig

CALIBRATION

ab195215 (lgG) - This immunoassy is calibrated against a highly purified human lgG. 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 lgG kit to approximate NIBSC International units, use the equation below.

NIBSC 67/086 approximate value (IU/mL) = 0.000017 (1.7e-5 IU) x SimpleStep IgG value (ng/mL)

Immunoglubulin G (IgG) is a glycoprotein molecule which belongs to the immunoglobulin family of proteins known as antibodies. Immunoglobulins are the key component of humoral immunity. IgG is a monomeric immunoglobulin, built of two heavy chains gamma and two light chains. The heavy chains are linked to each other and to the light chain by disulfide bonds. Each molecule has two antigen binding sites. While the amino-terminal portions that exhibit highly variable amino-acid composition are involved in antigen binding, the C terminal constant parts are involved in complement binding, placental passage and binding to cell membrane. This is the most abundant immunoglobulin and is approximately equally distributed in blood and in tissue liquids, constituting 75% of serum immunoglobulins in humans. This is the only isotype that can pass through the human placenta, thereby providing protection to the fetus in its first weeks of life before its own immune system has developed. It can bind to many kinds of pathogens, for example viruses, bacteria, and fungi, and protects the body against them by complement activation (classic pathway), opsonization for phagocytosis and neutralisation of their toxins. There are 4 subclasses: IgG1 (66%), IgG2 (23%), IgG3 (7%) and IgG4 (4%).

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Microplate

Platform

Properties

Storage instructions

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

Components	1 x 96 tests
10X Human lgG Capture Antibody	1 x 600µl
10X Human lgG Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml

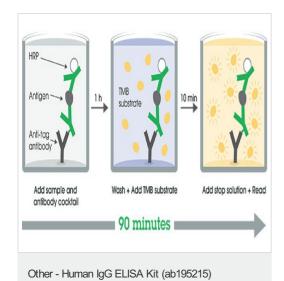
Notes

Components	1 x 96 tests
Antibody Diluent CP	1 x 6ml
Human IgG Lyophilized Purified Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

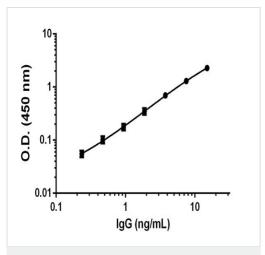
Cellular localization

Secreted

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 IgG standard curve in Sample Diluent NS.

,	Standard C	urve Measure	ments
Conc.	O.D. 4	450 nm	Mean
(ng/mL)	1	2	O.D.
0	0.07	0.07	0.07
0.23	0.13	0.12	0.13
0.46	0.18	0.16	0.17
0.93	0.26	0.23	0.25
1.87	0.45	0.38	0.42
3.75	0.79	0.75	0.77
7.5	1.44	1.30	1.37

Raw data values for example of Human IgG standard curve in Sample Diluent NS

2.26

2.45

2.35

15

O.D. (450 nm)	
0.1	1 10
	IgG (ng/mL)

Example of Human IgG standard curve in 1X Cell Extraction Buffer PTR.

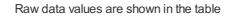
Background-subtracted data values (mean +/- SD) are graphed.

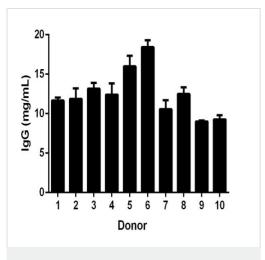
Raw data values are shown in the table

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

Standard Curve Measurements					
Conc.	O.D.	450 nm	Mean		
(ng/mL)	1	2	O.D.		
0	0.081	0.083	0.082		
0.23	0.14	0.13	0.13		
0.46	0.17	0.17	0.17		
0.93	0.26	0.25	0.25		
1.87	0.38	0.39	0.38		
3.75	0.69	0.67	0.68		
7.5	1.14	1.18	1.16		
15	1 79	1 96	1.87		

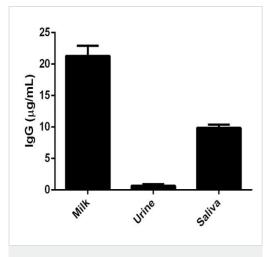
Raw data values for example of Human IgG standard curve in 1X Cell Extraction Buffer PTR





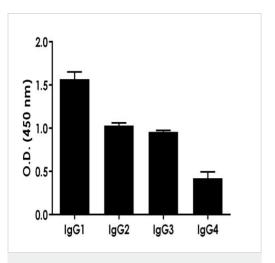
IgG levels in individual healthy donors.

Ten individual healthy donors were evaluated for the presence of $\lg G$ in serum using this assay. Results were interpolated from the standard curve in Sample Diluent NS and corrected for sample dilution (1:5x10⁶). The mean level of $\lg G$ was found at 12.5 mg/mL with a range of 9-18.4mg/mL.



Comparison of IgG levels in Human milk, urine and saliva.

Bodily fluids from 3 different donors were evaluated for the presence of lgG using this assay. Results were interpolated from the standard curve in sample diluent NS and corrected for sample dilution (1:2.5x10⁴). The mean levels in Milk were found at 20.7 μ g/mL, in Urine at 0.8 μ g/mL and in Saliva at 11.1 μ g/mL.



IgG1-4 isotypes are detected by this kit

Native lgG was measured in the human serum, plasma citrate, plasma EDTA, plasma heparin and culture media in a 2-fold dilution series. Sample dilutions were made in Sample Diluent NS for all

samples, purified IgG was spiked into culture media and diluted in a 2-fold dilution series in Sample Diluent NS

Human lgG1, lgG2, lgG3 and lgG4 were tested at 5ng/mL

Dilution Factor	Interpolated value	1:5x10 ⁶ Human Serum	1:2x10 ⁶ Human Plasma (Citrate)	1:2x10 ⁶ Human Plasma (EDTA)	1:4x10 ⁶ Human Plasma (Heparin)	1:10 Culture Media
-	ng/mL	3.46	7.02	5.88	4.59	8.35
1	% Expected value	100	100	100	100	100
_	ng/mL	1.82	3.37	2.92	2.17	4.03
2 %	% Expected value	105	96	99	95	96
- 2	ng/mL	0.86	1.69	1.54	1.14	1.77
4 %	% Expected value	99	96	105	99	86
	ng/mL	0.47	0.84	0.73	0.52	1.01
8	% Expected value	108	96	99	91	97
40	ng/mL	0.23	0.40	0.39	0.24	0.56
16	% Expected value	105	90	105	85	107

Linearity of dilution – native IgG in human serum, plasma (citrate, EDTA and Heparin), and culture media

Dilution Factor	Interpolated value	1:1x10 ³ Human Milk	1:50 Human Urine	1:500 Human Saliva	200 ng/mL HLH extract
4	ng/mL	16.9	16.65	17.96	19.16
1	% Expected value	100	100	100	100
_	ng/mL	7.21	7.88	9.74	9.89
2	% Expected value	85	95	108	103
.,	ng/mL	4.6	4.71	5.04	4.82
4	% Expected value	109	113	112	101
0	ng/mL	2.26	2.31	2.45	2.28
8	% Expected value	107	111	109	95
40	ng/mL	1.14	1.21	1.32	1.16
16	% Expected value	108	117	117	97

Linearity of dilution – native IgG in human milk, urine, saliva and HLH extract

Native IgG was measured in milk, saliva, urine and liver homogenate (HLH) in a 2-fold dilution series. Sample dilutions were made in Sample Diluent NS for all samples except for HLH, which was carried out in 1X cell extraction buffer PTR.

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