Version 4 Last updated 3 November 2021

ab229390
Human IgG CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of IgG in human serum, plasma, milk, saliva, urine, cell culture supernatants, and tissue extracts.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

IgG *in vitro* CatchPoint SimpleStep ELISA kit is designed for the quantitative measurement of IgG protein in human serum, plasma, milk, saliva, urine, cell culture supernatants, and tissue extracts.

The CatchPoint SimpleStep ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. CatchPoint HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.

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 has an approximate molecular weight of about 150kDa and it is composed of four peptide chains: two identical heavy chains (γ) of about 50kDa and two identical light chains (κ) of about 25kDa each. The heavy chains are linked to each other and to the light chain by disulfide bonds. At the N terminus, both the heavy and the light chain contain variable regions (VH and VL) which account for antibody diversity. At the C terminus, both chains contain constant regions (CH and CL) but only CH mediates effector functions.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 40 minutes

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 5 minutes

Read fluorescence at Ex/Cutoff/Em 530/570/590 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab195215).

6. Materials Supplied

Item	Quantity	Storage Condition
Human IgG Capture Antibody 10X	600 µL	+4°C
Human IgG Detector Antibody 10X	600 µL	+4°C
Human IgG Lyophilized Purified Protein	2 Vials	+4°C
Antibody Diluent CP	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H_2O_2, 3%)	50 µL	+4°C
Sample Diluent NS*	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

*Sample Diluent NS is provided but not necessary for this product.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The incubation times provided in this protocol were optimized for fastest results with good signal. It is possible to increase the signal with longer incubation times, further optimization might be necessary.
- Keep in mind any RFU values shown are relative, NOT absolute. RFU from one plate reader are not comparable to another, especially if different make or model.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

Special Handling Instructions for the Human IgG kit

IgG can bind to the surface of the skin microbiota. To prevent unintended background, it is recommended to clean bench surfaces and all pipettes to be used during the experiment with 10% bleach. Use a surgical mask and maintain gloves clean by either using 70% ethanol or by changing them frequently. Do not leave reagents or the plate opened while working or during assay incubation.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

  See Special Handling Instructions in section 8 before preparing reagents.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CP. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CP. Mix thoroughly and gently.

9.4 CatchPoint HRP Development Solution

Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer.

For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the IgG by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IgG standard by adding 1 mL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 50 ng/mL **Stock Standard** Solution.

For **serum, plasma, milk, urine, saliva, and cell culture supernatant samples** reconstitute the IgG standard sample by adding Sample Diluent NS.
For **tissue extract samples** reconstitute the Human IgG standard sample by adding 1X Cell Extraction Buffer PTR.

10.2 Label 12 tubes, Standards 1– 12.

10.3 Add 120 µL of appropriate diluent into tube number 1 and 150 µL of appropriate diluent into numbers 2-12.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #12 contains no protein and is the Blank control.

Standards will be added to the plate in step 13.3. If desired all 12 standards can be used for a full standard curve. Alternatively, to commit fewer wells to the standard curve, select a subset of at least 7 standards plus the blank control. If 7 standards are desired, we recommend standards #2-8.

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	180	120	50	30
2	Standard#1	150	150	30	15
3	Standard#2	150	150	15	7.5
4	Standard#3	150	150	7.5	3.75
5	Standard#4	150	150	3.75	1.88
6	Standard#5	150	150	1.88	0.94
7	Standard#6	150	150	0.94	0.47
8	Standard#7	150	150	0.47	0.23
9	Standard#8	150	150	0.23	0.12
10	Standard#9	150	150	0.12	0.06
11	Standard#10	150	150	0.06	0.03
12	None	0	150	0	0

11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Human Serum	$1:5x10^6 – 1:8x10^7$
Human Plasma - EDTA	$1:2x10^6 – 1:3x10^7$
Human Plasma - Citrate	$1:2x10^6 – 1:3x10^7$
Human Plasma - Heparin	$1:4x10^6 – 1:6x10^7$
Human Milk	1:1,000 – 1:10,000
Human Urine	1:50 – 1:500
Human Saliva	1:1,000 – 1:10,000
Human Cerebrospinal Fluid	1:2,000 – 1:32,000

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Urine:

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute in Sample Diluent NS and assay.

Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.5 Saliva:

Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.6 Milk:

De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples at least 1.5 X in Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

11.7 Cerebrospinal Fluid (CSF):

Dilute cerebrospinal fluid at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles

11.8 Preparation of extracts from cell pellets:

11.8.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.8.2 Rinse cells twice with PBS.

11.8.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.8.4 Incubate on ice for 20 minutes.

11.8.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.8.6 Transfer the supernatants into clean tubes and discard the pellets.

11.8.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.8.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.9 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.9.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.9.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.9.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.9.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.9.5 Transfer the supernatants into clean tubes and discard the pellets.

11.9.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.9.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.10 Preparation of extracts from tissue homogenates:

11.10.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.10.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.10.3 Incubate on ice for 20 minutes.

11.10.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.10.5 Transfer the supernatants into clean tubes and discard the pellets.

11.10.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.10.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Dilution of samples

Due to the high dilutions required for some samples, we recommend initially diluting your samples in 1X Wash Buffer and then performing the final dilution in Sample Diluent NS. The table below demonstrates the steps suggested to generate a final sample dilution of $1:2x10^6$. Ensure that the final dilution is equal or greater than 1:40 dilution factor to avoid a significant inadvertent dilution of the Sample Diluent NS.

Tube #	Sample to Dilute	Volume to Dilute (µL)	Volume of 1X Wash Buffer (µL)	Volume of Sample Diluent NS (µL)	Starting Conc.	Final Conc.
1	Neat	5	195	0	Neat	1:40
2	Tube #1	4	196	0	1:40	1:2,000
3	Tube #2	4	196	0	1:2,000	$1:1x10^5$
4	Tube #3	5	0	195	$1:1x10^5$	$1:2x10^6$

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well fluorescence or "edge effects" have not been observed with this assay.
- Ensure plate and all materials are equilibrated to room temperature during use.
- Cover the plate with a plate seal during incubation steps.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

See Special Handling Instructions in section 8 before proceeding with the assay.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

Note: Add antibody mixture to replicates at the same time to avoid well to well variation.

13.5 Seal the plate and incubate for 40 minutes at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 5 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.
Note: Add CatchPoint HRP Development Solution to replicates at the same time to avoid well to well variation.

13.8 Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org

Mode:	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

*For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

13.9 Analyze the data as described below.

14. Calculations

14.1 Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices

14.2 Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.

14.3 Create a standard curve by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

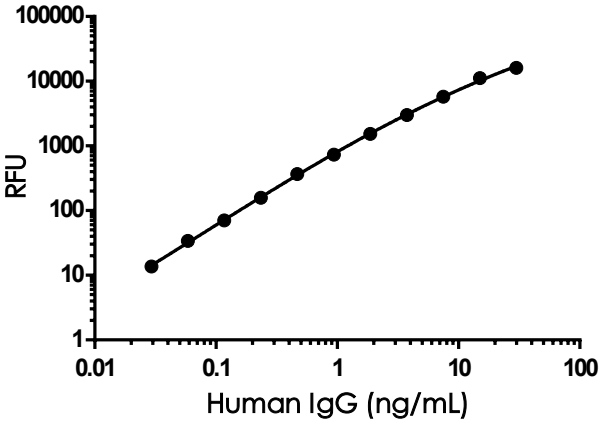
Δ **Note:** Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.4 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.5 Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (ng/mL)	RFU		Mean RFU
	1	2	
0	71	72	71
0.03	90	82	86
0.06	102	110	106
0.12	144	140	142
0.23	229	230	229
0.47	446	428	437
0.94	811	801	806
1.88	1,678	1,543	1,610
3.75	3,122	3,004	3,063
7.5	5,656	6,011	5,834
15	10,873	11,583	11,228
30	16,453	15,900	16,176

Figure 1. Example of human IgG standard curve in Sample Diluent NS. The IgG standard curve was prepared as described in Section 10. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 0.03 ng/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of IgG purified protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Human Serum (1:2x10^7)	101	88 – 125
Human Plasma - EDTA	90.17	87 – 93
Human Plasma - Citrate	100	98 – 102
Human Plasma - Heparin	100	100 – 100
Human Milk (1:8x10^4)	89	83 – 94
Human Urine (1:4x10^3)	87	82 – 93
Human Saliva (1:4x10^4)	89	80 – 106
Culture Media (1:10)	107	96 – 115
Human Cerebrospinal Fluid (1:4000)	98	88 – 105

Linearity of Dilution

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.

Native IgG was measured in the human serum, plasma citrate, plasma EDTA, plasma heparin, 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. Purified IgG was spiked into culture media and diluted in a 2-fold dilution series in Sample Diluent NS.

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
Undiluted	ng/mL	3.46	7.02	5.88	4.59	8.35
	% Expected value	100	100	100	100	100
2	ng/mL	1.82	3.37	2.92	2.17	4.03
	% Expected value	105	96	99	95	96
4	ng/mL	0.86	1.69	1.54	1.14	1.77
	% Expected value	99	96	105	99	86
8	ng/mL	0.47	0.84	0.73	0.52	1.01
	% Expected value	108	96	99	91	97
16	ng/mL	0.23	0.40	0.39	0.24	0.56
	% Expected value	105	90	105	85	107

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

PRECISION –

Mean coefficient of variations of interpolated values of IgG from three concentrations of human serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	6.4	14.7

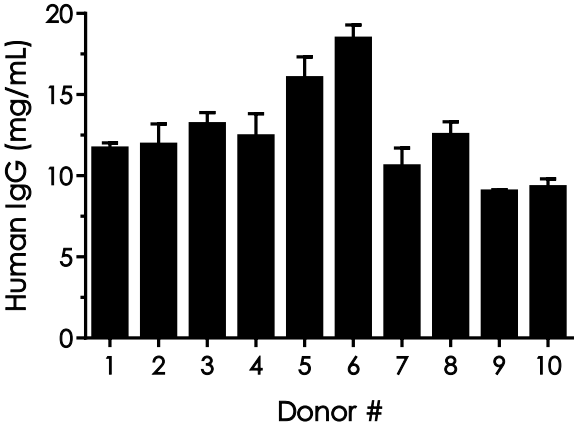


Figure 2. IgG levels in individual healthy donors. Ten individual healthy donors were evaluated for the presence of IgG in serum using this assay. Results were interpolated from the standard curve in Sample Diluent NS and corrected for sample dilution (1:5x10^6). The mean level of IgG was found at 12.5 mg/mL with a range of 9 – 18.4mg/mL.

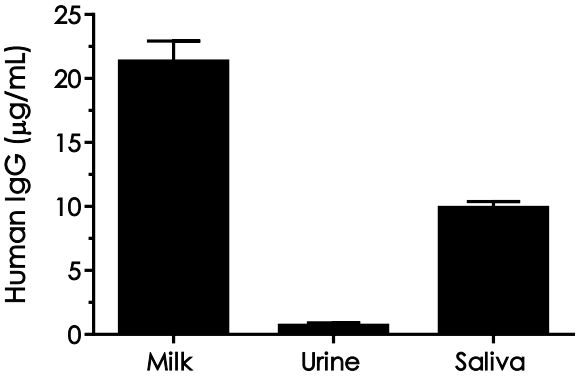


Figure 3. Comparison of IgG levels in human milk, urine and saliva. Bodily fluids from 3 different donors were evaluated for the presence of IgG using this assay. Results were interpolated from the standard curve in sample diluent NS and corrected for sample dilution (1:2.5x10^4). The mean levels in Milk were found at 20.7 µg/mL, in Urine at 0.8 µg/mL and in Saliva at 11.1 µg/mL.

17. Assay Specificity

This kit recognizes both native and recombinant human IgG protein in serum, plasma, and cell culture supernatant, cell and tissue extract samples only.

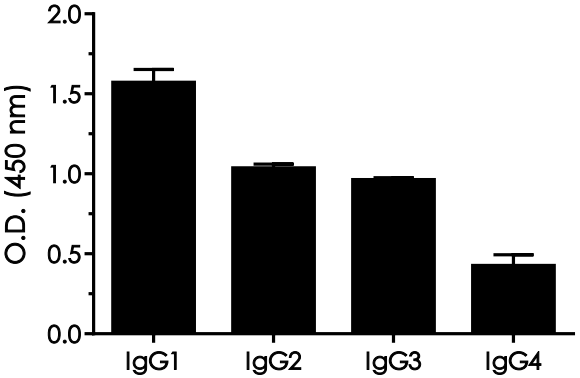


Figure 4. IgG1-4 isotypes are detected by this kit. Human IgG1, IgG2, IgG3 and IgG4 were tested at 5ng/mL.

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 IgM, human IgA and human IgE to determine interference. After background subtraction, recovery of human IgG was observed at a mean of 98% with a standard deviation of 0.06.

18. 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.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Guinea Pig
- Rabbit
- Dog
- Goat
- Sheep
- Cow

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer).
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with CatchPoint HRP Development Solution too brief	Read plate again after longer incubation time
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep Stoplight Red Substrate protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Note

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)