ab212169 – IgG Human SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of human IgG in 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.

Note: Sample incubation time has changed to 60 minutes.

Version 4b Last Updated 28 April 2025

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1. BACKGROUND

IgG *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IgG protein in human serum, plasma, milk, urine, saliva, culture media and tissue extracts.

The 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. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

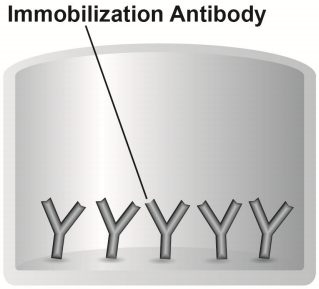
Immunoglubulin G (IgG) is a glycoprotein molecule which belongs to the immunoglobulin family of proteins known as antibodies. Immunoglobulins are the key component of humoral immunity. IgG has an approximate molecular weight of about 150kDa and it is composed of four peptide chains: two identical heavy 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. Structurally the IgG molecule may be divided into: (1) the Fragment antigen binding region (Fab) containing the VL, VH, CL and CH2 all of which shape the antigen

binding site and (2) the Fragment crystallizable region (Fc) containing CH domains 2 – 4 which stabilize the antibody and bind to the Fc receptor on the surface of macrophages, neutrophils, natural killer cells as well as to complement proteins to mediate therefore physiological effects.

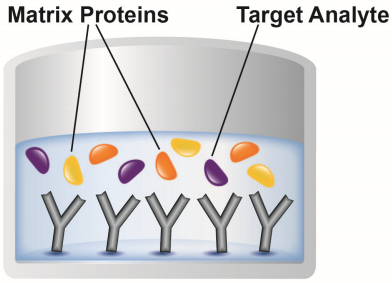
IgG is synthesized and secreted by plasma B cells in response to an immunogen after recognition of specific epitopes on the antigen and it is generated following class switching and maturation of an antibody response, thus providing immune protection. There are four subclasses of IgG in humans (IgG 1, 2, 3, 4) with variable affinity to Fc receptors and complement. The levels of IgG are generally considered to be indicative of an individual's immune status and are found increased in all types of infections, liver disease, severe malnutrition, dysproteinemia and rheumatoid arthritis. It is decrease in conditions such as hypogammaglobulinemia, X-linked agammaglobulinemia, lymphoid aplasia and chronic lymphoblastic leukemia. IgG accounts for 75% of the total human protein and can be found in serum, lymphatic fluid, cerebrospinal fluid, colostrum, milk, urine, saliva, sweat and body tissues. IgG has been shown to bind some bacterial strains from cutaneous microbiota.

The Fc portion of human IgG is frequently used as the basis of prolonged pharmacokinetics as it is used as a fusion partner to extend the half-life of fusion proteins.

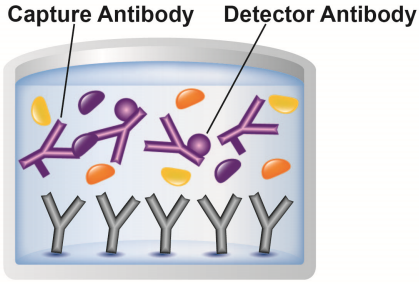
2. ASSAY SUMMARY



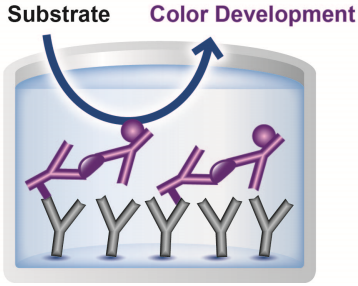
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Development Solution to each well and incubate. Add Stop Solution at a defined endpoint.

Alternatively, record color development kinetically after TMB substrate addition.

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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +4ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Human IgG Capture Antibody	6 mL	+4ºC
10X Human IgG Detector Antibody	6 mL	+4ºC
IgG Human Lyophilized Purified Protein	10 Vials	+4ºC
Antibody Diluent CP2	60 mL	+4ºC
10X Wash Buffer PT	200 mL	+4ºC
5X Cell Extraction Buffer PTR	50 mL	+4ºC
50X Cell Extraction Enhancer Solution	5 mL	+4ºC
TMB Development Solution	120 mL	+4ºC
Stop Solution	120 mL	+4ºC
Sample Diluent NS	2 x 250 mL	+4ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	10 x 96 Wells	+4ºC
Plate Seal	10	+4ºC

Note: Antibody Diluent CP2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CP previously used in this kit. While we run stock down, you may receive kits containing antibody diluent CP. This does not affect the way you should use the kit. If you have any questions please contact Abcam Scientific Support.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. 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.

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 provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4ºC. To dissolve, warm briefly at + 37ºC and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- **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

- Maintain bulk reagents at 4°C and remove the volume required for the day of experiment. Equilibrate this material to room temperature (18-25°C) prior to use. **The sample volumes below are sufficient for 96 wells (12 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 tissue extracts only)

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

Note – Do not add the 50X Cell Extraction Enhancer to the 1X Cell Extraction Buffer PTR as this may reduce the signal of standards and samples but as high as 10%.

9.2 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 100 mL 1X Wash Buffer PT combine 10 mL 10X Wash Buffer PT with 90 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CP2. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent CP2. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use. The following table describes the preparation of a standard curve for duplicate measurements (recommended)

See Special Handling Instructions in section 8 before preparing standards.

10.1 IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the Human IgG standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Human IgG standard by adding 1000 µL Diluent.

For **serum, plasma, milk, urine, saliva and culture media measurements**, reconstitute the Human IgG protein by adding Sample Diluent NS.

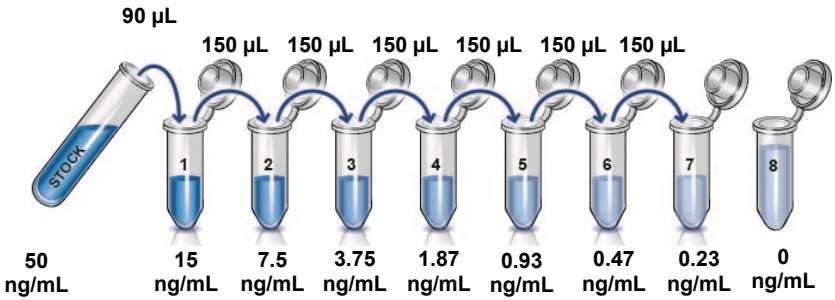
For **tissue extract measurements**, reconstitute the Human IgG protein by adding 1X Cell Extraction Buffer PTR.

Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. This is the 50 ng/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 210 µL of appropriate sample buffer (see Step 10.1) into tube number 1 and 150 µL of appropriate sample buffer into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

See Special Handling Instructions in section 8 before preparing samples.

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

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 -20ºC 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 -20ºC 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 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 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 dilute in Sample Diluent NS and assay. Store 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 4ºC and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4ºC and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples in Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.7 **Preparation of extracts from tissue homogenates**

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

11.7.2 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.

11.7.3 Incubate on ice for 20 minutes.

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

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

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

11.7.7 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 absorbance or “edge effects” have not been observed with this assay.

13. ASSAY PROCEDURE

- **Maintain stock reagents at 4°C and only equilibrate to room temperature the volume of reagent necessary.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

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 **60** 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. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark (avoiding signal saturation) on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step

before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well in the same order as the TMB was added to avoid well to well variation. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.9 Analyze the data as described below.

14. CALCULATIONS

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

14.2 Create a standard curve by plotting the average blank control subtracted absorbance 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.

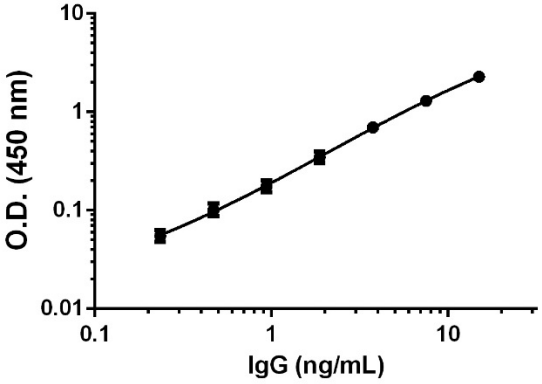
Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better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

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance 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.

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

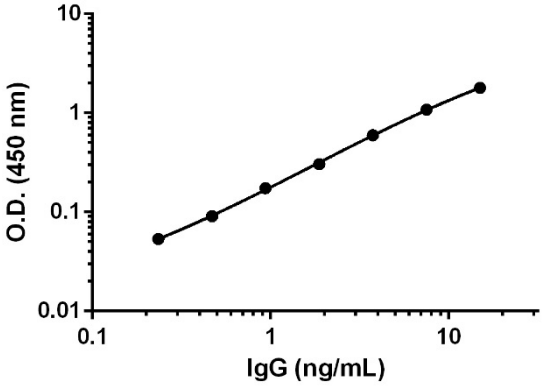
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (ng/mL)	O.D. 450 nm		Mean O.D.
	1	2	
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
15	2.45	2.26	2.35

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



Standard Curve Measurements			
Conc. (ng/mL)	O.D. 450 nm		Mean O.D.
	1	2	
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

Figure 2. Example of human IgG standard curve in 1X Cell Extraction Buffer PTR. The human IgG standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 20 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentrations.

RECOVERY –

Three concentrations of IgG 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 (1:4x10^6)	90.17	87 – 93
Human Plasma - Citrate (1:4x10^6)	100	98 – 102
Human Plasma - Heparin (1:8x10^6)	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

LINEARITY OF DILUTION –

Linearity of dilution was 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
1	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 from 3 concentrations of Serum within the working range of the assay.

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

SAMPLE DATA –

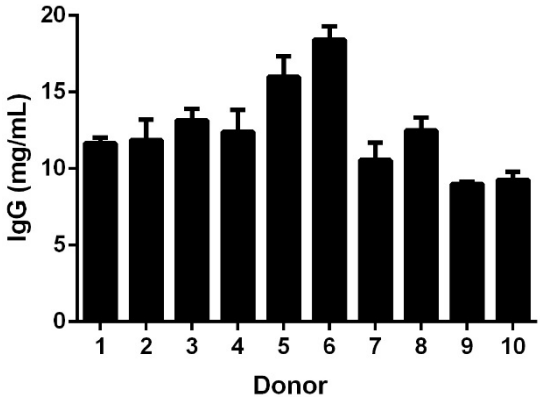


Figure 3. IgG levels in individual healthy donors**.** Ten individual healthy donors were evaluated for the presence of IgG in serum using this assay. Results were interpolated from the standard curve in Sample Diluent NS and corrected for sample dilution ($1:5x10^6$). The mean level of IgG was found at 12.5 mg/mL with a range of 9 – 18.4mg/mL.

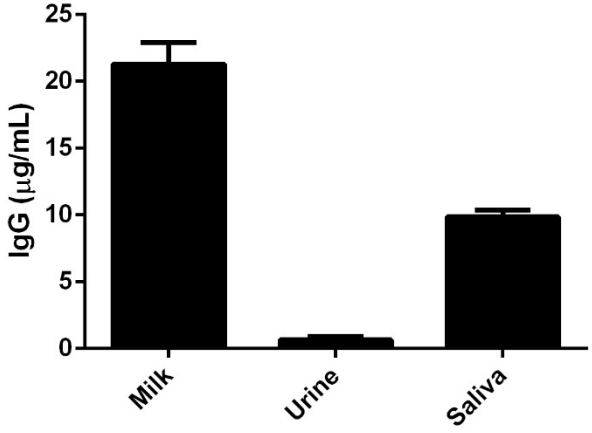


Figure 4. Comparison of IgG levels in human milk, urine and saliva. Bodily fluids from 3 different donors were evaluated for the presence of IgG using this assay. Results were interpolated from the standard curve in sample diluent NS and corrected for sample dilution (1:2.5x10^4). The mean levels in 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 purified human IgG protein in serum, plasma, milk, urine, saliva, cell culture media, and tissue extracts samples only.

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	Cause	Solution
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 TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
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 TMB Development Solution 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 37ºC.

20. **NOTES**

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)