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Human IgG2 SimpleStep ELISA® Kit

For the quantitative measurement of IgG2 in human serum, plasma, milk, urine, saliva and cell culture supernatants.

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

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1. Overview

Human IgG2 *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IgG2 protein in human serum, plasma, milk, urine, saliva and cell culture supernatants.

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.

Immunoglobulin 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 decrease in conditions such as hypogammaglobulinemia and X-linked agammaglobulinemia. IgG accounts for 75% of the total human protein and can be found in serum, lymphatic fluid, cerebrospinal fluid, colostrum, milk, urine, saliva, tissues, sweat and skin sebum.

IgG2, the second largest part of IgG isotypes in humans, comprises 20-25% of the main subclass and is the prevalent immune response against carbohydrate-/polysaccharide antigens. Among all IgG isotypes, a deficiency in IgG2 is the most common one and associated with recurring airway/respiratory infections caused by encapsulated bacteria such as pneumococci and/or Haemophilus influenza type B.

2. Protocol Summary

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 Development Solution 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.
- 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 handled 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.

6. Materials Supplied

Item	Quantity	Storage Condition
Human IgG2 Capture Antibody (Lyophilized)	1 vial	4°C
10X Human IgG2 Detector Antibody	600 µL	4°C
Human IgG2 Lyophilized Purified Protein	2 vials	4°C
Antibody Diluent CPI2	6 mL	4°C
10X Wash Buffer PT	20 mL	4°C
TMB Development Solution	12 mL	4°C
Stop Solution	12 mL	4°C
Sample Diluent NS	50 mL	4°C
SimpleStep Pre-Coated 96-Well Microplate	1 unit	4°C
Plate Seals	1 unit	4°C

Note: Antibody Diluent CPI2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CPI previously used in this kit.
While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit.
If you have any questions please contact Abcam Scientific Support.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform 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 KH_2PO_4, 8 mM Na_2HPO4, 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).

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.
- 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.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

Δ Note: 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.

Δ Note: Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 10X Wash Buffer PT

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

9.2 Human IgG2 Capture Antibody (Lyophilized)

To reconstitute the lyophilized IgG2 capture antibody, centrifuge the vial at 10,000 x *g* for 2 minutes and then add 660 µL of Sample Diluent NS per vial. Incubate at room temperature for 5 minutes and mix thoroughly ensuring the material is fully resuspended.
The 10X IgG2 Capture Antibody may be stored at -20°C. Avoid repeat freeze/thaw cycles.

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Human IgG2 Detector Antibody with 2.4 mL Antibody Diluent CPI. 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 IgG2 standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IgG2 standard by adding 0.5 mL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 600 ng/mL Stock Standard Solution.

10.2 Label eight tubes #1 – 8.

10.3 Add 250 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2 - 8.

10.4 To prepare **Standard #1** add 50 µL of the 600 ng/mL IgG2 Stock Standard to tube #1.

10.5 Add 150 µL from **Standard #1** into tube #2 to create **Standard #2**.

10.6 Using the table below as a guide, prepare subsequent serial dilutions. Sample Diluent NS serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume Diluent NS (µL)	Human IgG2 (ng/mL)
1	50 µL Stock Standard	250	100
2	150 µL Standard #1	150	50
3	150 µL Standard #2	150	25
4	150 µL Standard #3	150	12.5
5	150 µL Standard #4	150	6.25
6	150 µL Standard #5	150	3.12
7	150 µL Standard #6	150	1.56

8 (Blank)	N/A	150	0

11. Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	Range
Human serum	1:300,000 – 1:1,200,000
Human plasma – EDTA	1:200,000 – 1:1,600,000
Human plasma – Heparin	1:300,000 – 1:2,400,000
Human plasma – Citrate	1:300,000 – 1:2,400,000
Human milk	1:800 – 1:6,400
Human urine	1:30 – 1:240
Human saliva	1:400 – 1:1,600
Cell culture media	1:10 – 1:160

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 dilute samples into

Sample Diluent NS 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 and dilute samples into 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 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 in Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

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

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

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 4°C 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.

13.5 Seal the plate and incubate for 1 hour 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 3 minutes in the dark on a plate shaker set to 400 rpm.
Given variability in laboratory environmental conditions, optimal incubation time may vary between 3 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. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative: Instead of the endpoint reading at 450 nm, record the development of TMB Development Solution 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 minutes
Interval:	20 secs - 1 minute
Shaking:	Shake between readings

Δ Note: The 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.

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.

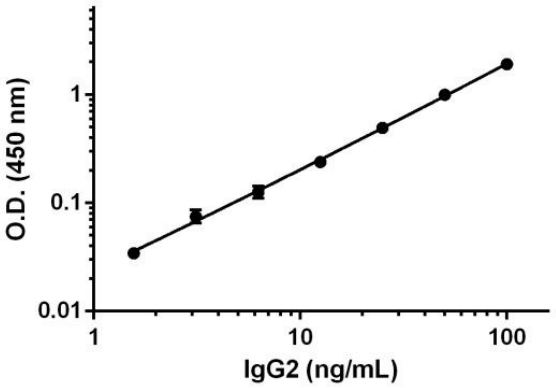
Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a 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.	O.D. 450 nm		Mean
(ng/mL)	1	2	O.D.
0	0.115	0.113	0.115
1.56	0.160	0.162	0.161
3.12	0.194	0.209	0.202
6.25	0.242	0.264	0.253
12.5	0.371	0.359	0.365
25	0.615	0.625	0.620
50	1.098	1.148	1.123
100	2.076	2.000	2.038

Figure 1. Example of IgG2 standard curve in sample diluent NS. The IgG2 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 750 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=28) and adding 2 standard deviations then extrapolating the corresponding concentration.

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of Serum within the working range of the assay

	Intra-assay Precision	Inter-Assay Precision
n =	8	3
CV (%)	5.3	4.3

RECOVERY –

Three concentrations of IgG2 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 (1:600,000)	99.7%	97.4 – 102.4
Human Plasma - EDTA (1:600,000)	102.5%	98 - 105
Human Plasma - Heparin	101.3%	97.4 - 107.4
Human Plasma - Citrate (1:600,000)	107.5%	103 - 110
Human Milk (1:200,000)	90%	86 - 94
Human Urine (1:100)	96.5%	90 - 101
Human Saliva (1:1000)	104%	93 - 124

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 IgG2 was measured in Human serum, plasma citrate, plasma EDTA, plasma heparin, milk, urine and saliva in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Purified IgG2 was spiked into cell culture media and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	1:300,000 Human Serum	1:300,000 Human Plasma (Citrate)	1:200,000 Human Plasma (EDTA)	1:300,000 Human Plasma (Heparin)
Undiluted	ng/mL	11.7	11.3	10.0	13.2
	% Expected value	100	100	100	100
2	ng/mL	5.9	5.6	5.8	7.4
	% Expected value	101	100	115	111
4	ng/mL	2.6	2.4	2.6	4.0
	% Expected value	90	86%	10	121
8	ng/mL	-	1.5	1.1	1.4
	% Expected value	NL	105	89	84

Dilution Factor	Interpolated value	1:800 Human Milk	1:30 Human Urine	1:400 Human Saliva	1:10 Culture media
Undiluted	ng/mL	10.03	14.6	7.5	47.0
	% Expected value	100	100	100	100
2	ng/mL	6.63	8.3	3.6	24.5
	% Expected value	123	114	96	104
4	ng/mL	3.15	4.2	1.6	12.17
	% Expected value	116	115	86	104
8	ng/mL	1.3	2.0	-	5.95
	% Expected value	96	110	NL	101
16	ng/mL	-	-	-	3.2
	% Expected value	NL	NL	NL	110

NL = Non-linear

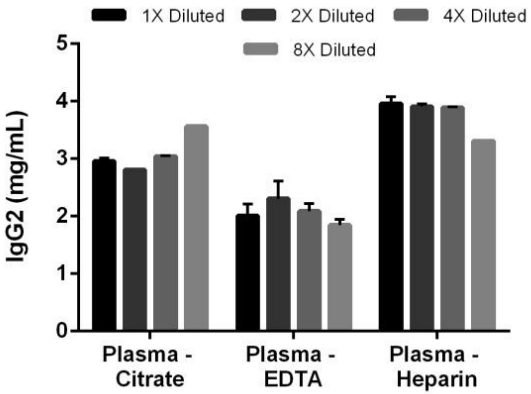


Figure 2. Linearity of dilution of native IgG2 in human citrate, EDTA, and heparin plasmas. Native human IgG2 protein was measured in citrate plasma (1:300,000), EDTA plasma (1:200,000), and heparin plasma (1:300,000) in a 2-fold dilution series in Sample Diluent NS. The interpolated dilution factor corrected values are graphed (mean +/- SD).

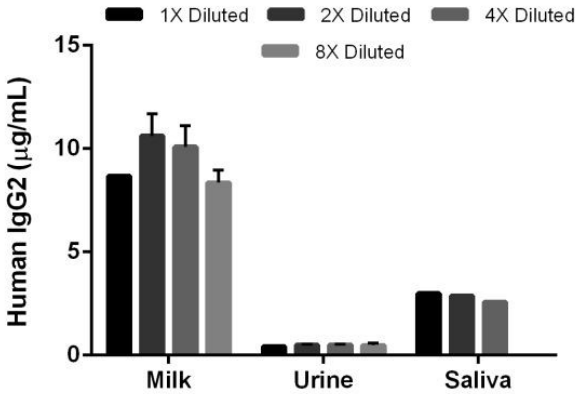


Figure 3. Linearity of dilution of native IgG2 in human milk, urine and saliva. Native human IgG2 protein was measured in milk (1:800), urine (1:30), and saliva (1:400) in a 2-fold dilution series in Sample Diluent NS. The interpolated dilution factor corrected values are graphed (mean +/- SD).

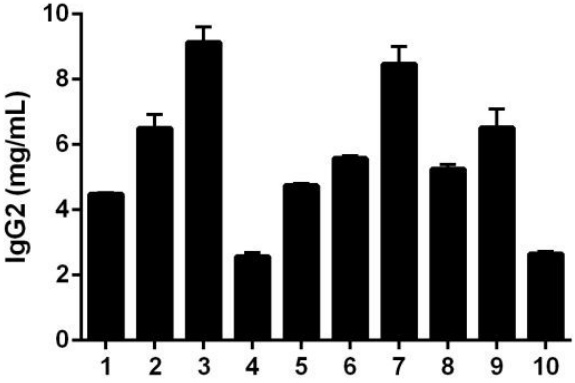


Figure 4. Levels of IgG2 in human donors. Ten individual healthy male donors were evaluated for the presence of IgG2 in serum using this assay. Results were interpolated from the standard curve in Sample Diluent NS and corrected by sample dilution (1:500,000). In the 10 individual donors, the mean level of IgG2 is 5.6 mg/mL with a range of 2.5 – 9.1 mg/mL and a standard deviation of 2.16 mg/mL.

17. Assay Specificity

This kit recognizes both native and purified human IgG2 protein in serum, plasma, milk, urine, saliva and culture supernatants.

CROSS REACTIVITY

The purified proteins listed below were prepared at 250 ng/mL in sample Diluent NS and assayed for cross reactivity.

Human purified proteins: IgA, IgM, IgE, IgG, IgG3, IgG4

Mouse purified proteins: IgG1, IgG2a, IgG2b, IgG3

No significant cross reactivity was observed with a mean OD deviation from background of 0.011.

INTERFERENCE

Purified human IgG2 was assayed at 20 ng/mL in the presence and absence of 250 ng/mL of human IgA, human IgE, mouse IgG1, mouse IgG2a, mouse IgG2b, and mouse IgG3 to determine interference.

After background subtraction, recovery of human IgG2 in the presence of these proteins was observed at a mean of 98.3% with a standard deviation of 1.5%.

Purified human IgG2 was assayed at 20ng/mL in the presence and absence of multiple concentrations of IgG1, IgG3 and IgG4 to determine interference.

The expected % recovery of human IgG2 is shown below:

Human IgG1 (ng/mL)	Expected % recovery of IgG2
250	20
120	23
60	56
30	73
15	93

Human IgG3 (ng/mL)	Expected % recovery of IgG2
250	26
120	39
60	56
30	79
15	100

Human IgG4 (ng/mL)	Expected % recovery of IgG2
250	53
120	62
60	94
30	99
15	100

18. Species Reactivity

This kit recognizes human IgG2 protein.

Other species reactivity was determined by measuring 1:150,000 diluted 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.

Species	Cross reactivity
Mouse	<3%
Rat	<3%
Guinea Pig	<3%
Rabbit	<3%
Dog	<3%
Goat	<3%
Pig	<3%
Cow	<3%

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	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 - 3 hours 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 37°C.

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)