ab326310 – Human IgG2 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of IgG2 in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, saliva, urine, and milk.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326310

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (ab202402).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human IgG2 Lyophilized Capture Antibody	1 Vial	+4°C
Human IgG2 Detector Antibody 10X	600 µL	+4°C
Human IgG2 Lyophilized Purified Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

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.

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.

10X Capture Antibody: To reconstitute the lyophilized capture antibody, centrifuge at 10,000 g for 2 minutes. Add 660 µL of Sample Diluent NS, let sit at room temperature for 5 minutes and resuspend well by inverting the tube by hand and gently pipetting. Unused reconstituted antibody can be stored frozen at -20°C. Avoid repeated freeze-thaw cycles.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. 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 CPI2. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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).

1. Reconstitute the IgG2 standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 600 ng/mL **Stock Standard #1** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 150 µL of Sample Diluent NS into tube numbers 2-8.
4. Use the **Stock Standard #1** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	**Stock Standard#1**	225	0	600	600
2	**Stock Standard#1**	75	150	600	200
3	Standard#2	75	150	200	66.67
4	Standard#3	75	150	66.67	22.22
5	Standard#4	75	150	22.22	7.41
6	Standard#5	75	150	7.41	2.47
7	Standard#6	75	150	2.47	0.82
8	Blank Control	0	150	0	0

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

*Media is RPMI 1640 containing 10% fetal bovine serum.

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.
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.
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 samples into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.
Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatantds, and dilute samples into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.
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 un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.
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 un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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.

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.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
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.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
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 30 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.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

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

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support
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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human IgG2.

The standard protein in this kit is the full length heavy constant gamma chain 2 of human IgG2.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, urine, saliva, and milk sample types.

CSF, cell extract, and tissue extract samples have not been tested with this kit.

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 20 ng/mL in the presence and absence of multiple concentrations of IgG1, IgG3, and IgG4 to determine interference.

The expected % recover 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

SPECIES REACTIVITY

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.

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

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence 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 chemiluminescence 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.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence 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.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

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)	RLU		Mean RLU
	1	2	
0	18,668	18,668	18,668
0.82	33,234	33,352	33,293
2.47	64,083	62,807	63,445
7.41	161,800	163,220	162,510
22.22	414,350	413,750	414,050
66.67	1,143,000	1,067,900	1,105,450
200	2,652,600	2,566,900	2,609,750
600	4,503,900	4,188,500	4,346,200

Table 1. Example of human IgG2 standard curve in Sample Diluent NS. The IgG2 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 284 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

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)	100	97 - 102
Human Plasma - EDTA (1:600,000)	103	98 - 105
Human Plasma - Heparin (1:600,000)	101	97 - 107
Human Plasma - Citrate (1:600,000)	108	103 - 110
Human Milk (1:200,000)	90	86 - 94
Human Urine (1:100)	97	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 the following biological samples in a 2-fold dilution series. Sample dilutions are made 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**

Native IgG2 was measured in the following biological samples (milk, urine, and saliva) in a 2-fold dilution series. Purified IgG2 was spiked into cell culture media and diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

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

Precision

Mean coefficient of variations of interpolated values of IgG2 from three concentrations of serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.3	4.3

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support
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