ab325734 – Human IgG3 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of IgG3 in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, urine, saliva, 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/ab325734

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 (ab318929).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human IgG3 Capture Antibody Lyophilized	1 Vial	10 x 1 Vial	+4°C
Human IgG3 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IgG3 Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent CP2	6 mL	10 x 6 mL	+4°C
Sample Diluent NS	12 mL	2 x 50 mL	+4°C
Wash Buffer PT 10X	2 x 20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+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 10 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 CP2. 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 CP2. 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 IgG3 standard sample by adding the volume of 1X Wash Buffer PT indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 160 ng/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 212.5 µL of 1X Wash Buffer PT into tube number 1 and 150 µL of 1X Wash Buffer PT into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	187.5	212.5	160,000	75,000
2	Standard#1	75	150	75,000	25,000
3	Standard#2	75	150	25,000	8,333
4	Standard#3	75	150	8,333	2,778
5	Standard#4	75	150	2,778	925.9
6	Standard#5	75	150	925.9	308.6
7	Standard#6	75	150	308.6	102.9
8	Standard#7	75	150	102.9	34.3
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:800,000 - 1:50,000
Plasma – Citrate	1:800,000 - 1:50,000
Plasma – EDTA	1:800,000 - 1:50,000
Plasma – Heparin	1:800,000 - 1:50,000
Urine	0.63 - 10%
Saliva	0.16 - 2.5%
Milk	0.25 - 4%
Cell Culture Supernatant	≤ 50%

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 at least 1:50,000 into 1X Wash Buffer PT and assay. Store un-diluted serum at -20ºC 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 at least 1:50,000 into 1X Wash Buffer PT and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. 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 Dilute samples at least 1:2 into 1X Wash Buffer PT and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:10 into 1X Wash Buffer PT and assay. Store un-diluted urine samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:40 into 1X Wash Buffer PT and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

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 samples at least 1:25 into 1X Wash Buffer PT and assay. Store un-diluted de-fatted milk at -20ºC 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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Version 2a | 2026-02-16

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human IgG3.

The standard protein in this kit is a recombinant fragment of human IGHG3 (Uniprot P01860) that is calibrated to a natively purified human IgG3 protein.

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

Spiked protein experiments were used to validate cell culture supernatant sample types.

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

CROSS REACTIVITY

50 ng/mL of native human IgG1 IgG2, IgG4, IgA, IgD, IgE, and IgM were tested individually for cross-reactivity. Cross reactivity is as follows:

Native Protein	Cross Reactivity
Human IgG1	0%
Human IgG2	0%
Human IgG4	0.33%
Human IgA	0%
Human IgD	0%
Human IgE	0%
Human IgM	0%

INTERFERENCE

850 pg/mL of recombinant human IgG3 and 50 ng/mL of the following native human immunoglobulins were tested in pairs for interference: IgG1, IgG2, IgG4, IgA, IgD, IgE, and IgM. No interference was observed.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 1:100,000 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.

No signal was observed for the following species: Monkey, Mouse, Rat, Cow, Pig, Rabbit, Goat, Dog

Additionally, media with 10% fetal bovine serum (FBS) was tested and no signal was observed.

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 (pg/mL)	RLU		Mean RLU
	1	2	
0	2,821	2,821	2,821
34.3	5,725	4,304	5,015
102.9	10,502	8,461	9,482
308.6	24,859	20,705	22,782
925.9	67,588	64,477	66,033
2,778	219,590	209,660	214,625
8,333	689,480	716,050	702,765
25,000	2,168,300	2,119,600	2,143,950
75,000	7,504,100	7,036,100	7,270,100

Table 1. Example of human IgG3 standard curve in 1X Wash Buffer PT. The IgG3 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 22.7 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 IgG3 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 (%)
1:200,000 Serum	100	96 - 108
1:200,000 Plasma – Citrate	108	103 - 115
1:200,000 Plasma – EDTA	106	101 - 113
1:200,000 Plasma – Heparin	107	98 - 118
1% Urine	96	94 - 98
0.75% Saliva	103	97 - 112
1% Milk	109	95 - 125
50% Cell Culture Media* Containing 10% FBS	78	68 – 85
50% Serum-free Cell Culture Media*	85	76 - 95

*Cell culture media is DMEM

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 IgG3 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Wash Buffer PT

Dilution Factor	Interpolated value	1:50,000 Human Serum	1:50,000 Human Plasma (Citrate)	1:50,000 Human Plasma (EDTA)	1:50,000 Human Plasma (Heparin)
Undiluted	pg/mL	10,376.7	9,160.5	8,350.0	9,123.2
	% Expected value	100	100	100	100
2	pg/mL	5,254.2	4,724.7	4,056.5	4,549.3
	% Expected value	101	103	97	100
4	pg/mL	2,731.5	2,363.6	2,194.4	2,391.0
	% Expected value	105	103	105	105
8	pg/mL	1,315.2	1,125.1	1,062.5	1,133.1
	% Expected value	101	98	102	99
16	pg/mL	641.8	542.5	502.9	565.9
	% Expected value	99	95	96	99

Native IgG3 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Wash Buffer PT.

Dilution Factor	Interpolated value	10% Human Urine	2.5% Human Saliva	4% Human Milk
Undiluted	pg/mL	3,747.2	7,615.0	10,784.2
	% Expected value	100	100	100
2	pg/mL	2,030.9	3,870.8	5,233.3
	% Expected value	108	102	97
4	pg/mL	1,010.2	1,789.2	2,876.0
	% Expected value	108	94	107
8	pg/mL	503.8	918.2	1,418.7
	% Expected value	108	96	105
16	pg/mL	257.7	435.7	707.4
	% Expected value	110	92	105

Recombinant IgG3 was spiked into the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Wash Buffer PT.

Dilution Factor	Interpolated value	50% Cell Culture Media w/FBS	50% Serum-free Cell Culture Media
Undiluted	pg/mL	5,735.9	5,037.2
	% Expected value	100	100
2	pg/mL	3,013.6	2,881.0
	% Expected value	105	114
4	pg/mL	1,654.7	1,470.7
	% Expected value	115	117
8	pg/mL	841.6	740.8
	% Expected value	117	118
16	pg/mL	441.3	379.6
	% Expected value	123	121

Precision

Mean coefficient of variations of interpolated values of IgG3 from a single concentration of serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.3	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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Version 2a | 2026-02-16

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