Human IgG1 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human IgG1

Catalog Numbers BMS2092 or BMS2092TEN

Pub. No. MAN0016537 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Human IgG1 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human IgG1.

Summary

IgG is the major immunoglobulin in blood, lymph fluid, cerebrospinal fluid, and peritoneal fluid and a key player in the humoral immune response. Serum IgG in healthy humans presents approximately 15% of total protein beside albumins, enzymes, other globulins and many more.

The Fc portion of IgG, but not F(ab´)2 or Fab fragments, can cross the placenta of a mother to enter the fetal circulation providing the fetus with postpartum protection.

IgG molecules are able to react with Fc γ receptors that are present on the surfaces of macrophages, neutrophils, natural killer cells, and can activate the complement system.

The binding of the Fc portion of IgG to the receptor present on a phagocyte is a critical step in the opsonizing property IgG provides to the immune response. Phagocytosis of particles coated with IgG antibodies is a vital mechanism to cope with microorganisms.

IgG is produced in a delayed response to an infection and can be retained in the body for a long time. The longevity in serum makes IgG most useful for passive immunization by transfer of this antibody. Detection of IgG usually indicates a prior infection or vaccination.

IgG1 comprises 60–65% of the total main subclass IgG, and is predominantly responsible for the thymus mediated immune response against proteins and polypeptide antigens. IgG1 binds to the Fc-receptor of phagocytic cells and can activate the complement cascade via binding to C1 complex. IgG1 immune response can already be measured in new borns and reaches its typical concentration in infancy. A deficiency in IgG1 isotype typically is a sign of a Hypogammaglobulinemia.

For literature update refer to our website.

Principles of the test

An anti-human IgG1 coating antibody is adsorbed onto microwells.

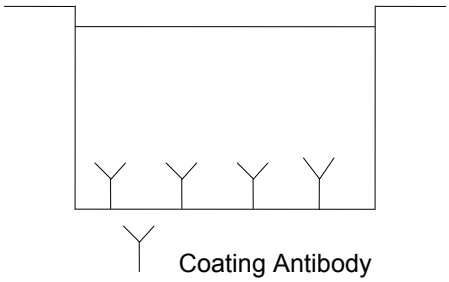

Fig. 1 Coated microwell

Human IgG1 present in the sample or standard binds to antibodies adsorbed to the microwells and a HRP-conjugated anti-human IgG antibody is added and binds to human IgG1 captured by the first antibody.

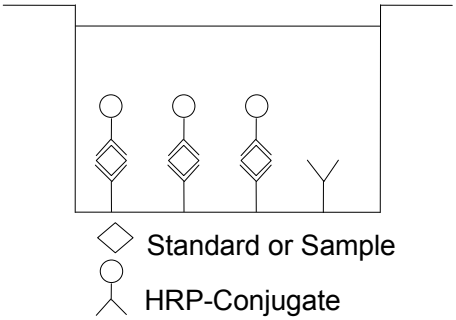

Fig. 2 First incubation

Following incubation unbound HRP-conjugated anti-human IgG antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

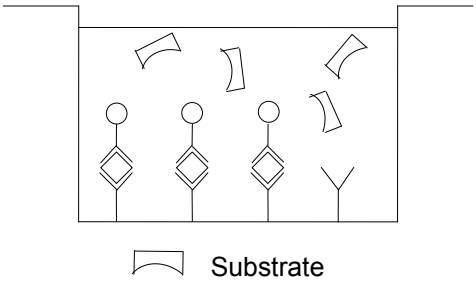

Fig. 3 Second incubation

A colored product is formed in proportion to the amount of human IgG1 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IgG1 standard dilutions and human IgG1 sample concentration determined.

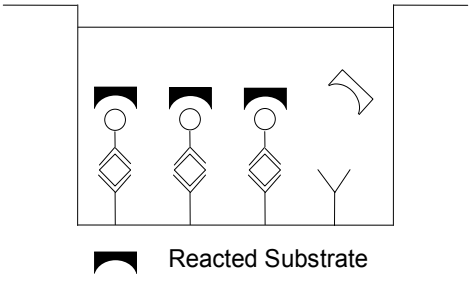

Fig. 4 Third incubation

Reagents provided

Reagents for human IgG1 ELISA BMS2092 (96 tests)

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human IgG1

1 vial (70 µL) HRP-Conjugate anti-human IgG monoclonal antibody

2 vials human IgG1 Standard lyophilized, 2000 ng/mL upon reconstitution

2 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)

1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

2 Adhesive Films

Reagents for human IgG1 ELISA BMS2092TEN(10x96 tests)

10 aluminum pouches with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human IgG1

10 vials (70 µL) HRP-Conjugate anti-human IgG monoclonal antibody

10 vials human IgG1 Standard lyophilized, 2000 ng/mL upon reconstitution

18 vials (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)

2 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

10 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Serum and plasma (citrate, heparin, EDTA) were tested with this assay. Other biological samples might be suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Pay attention to a possible *Hook Effect* due to high sample concentrations (see "Calculation of results" on page 4)

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human IgG1. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (for stability refer to "Sample stability" on page 6). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

1. Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2°C to 25°C. The Wash Buffer (1x) is stable for 30 days.
3. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

Assay buffer (1x)

1. Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
2. Store at 2°C to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
3. Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	5.0	95.0
1 - 12	10.0	190.0

HRP-Conjugate

Note: The HRP-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated HRP-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Human IgG1 standard

1. Reconstitute human IgG1 standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 2000 ng/mL).
2. Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

The standard has to be used immediately after reconstitution and cannot be stored.

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 2-fold serial dilutions for the standard curve as follows: Pipette 225 µL of Assay Buffer (1x) into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 2000 ng/mL) into the first tube, labeled S1, and mix (concentration of S1 = 1000 ng/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 5).

   Assay Buffer (1x) serves as blank.

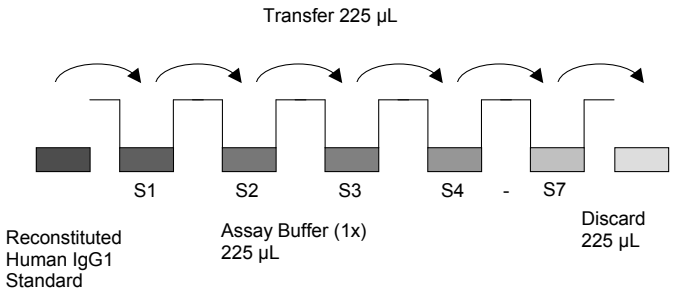

Fig. 5 Dilute standards - tubes

Test protocol

Note: Shaking is absolutely necessary for an optimal test performance.

1. Predilute your samples before starting with the test procedure. Dilute serum and plasma samples 1:10,000 with Assay Buffer (1x) according to the following scheme:

   10 µL sample + 990 µL Assay Buffer (1x) = Predilution A

   10 µL Predilution A + 990 µL Assay Buffer (1x) = Predilution B
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°C to 8°C sealed tightly.
3. Prepare HRP-conjugated antibody (see "HRP-Conjugate" on page 3).
4. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
5. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see "External standard dilution" on page 3):

   Add 100 µL of Assay Buffer (1x) in duplicate to all standard wells. Pipette 100 µL of prepared standard (see "Human IgG1 standard" on page 3, concentration = 2000 ng/mL), in duplicate, into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 1000 ng/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 6). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human IgG1 standard dilutions, ranging from 1000 ng/mL to 15.6 ng/mL. Discard 100 µL of the contents from the last microwells ([last microwells]) used.

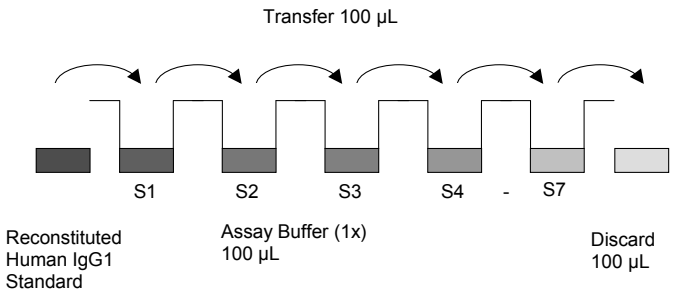

Fig. 6 Dilute standards - microwell plate.

In case of an external standard dilution (see "External standard dilution" on page 3), pipette 100 µL of these standard dilutions (S1–S7) in the standard wells according to Table 1.

Table 1 Example of the arrangement of blanks, standards and samples in the microwell strips.

	1	2	3	4
A	Standard 1 1000 ng/mL	Standard 1 1000 ng/mL	Sample 1	Sample 1
B	Standard 2 500 ng/mL	Standard 2 500 ng/mL	Sample 2	Sample 2
C	Standard 3 250 ng/mL	Standard 3 250 ng/mL	Sample 3	Sample 3
D	Standard 4 125 ng/mL	Standard 4 (125 ng/mL)	Sample 4	Sample 4
E	Standard 5 62.5 ng/mL	Standard 5 62.5 ng/mL	Sample 5	Sample 5
F	Standard 6 31.3 ng/mL	Standard 6 31.3 ng/mL	Sample 6	Sample 6
G	Standard 7 15.6 ng/mL	Standard 7 15.6 ng/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

6. Add 100 µL of Assay Buffer (1x) in duplicate to the blank wells.
7. Add 50 µL of Assay Buffer (1x) to the sample wells.
8. Add 50 µL of each prediluted sample in duplicate to the sample wells.
9. Add 50 µL of diluted HRP-conjugated antibody to all wells, including the blank wells.
10. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 1 hour on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance.)
11. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 3. of the test protocol. Proceed immediately to the next step.
12. Pipette 100 µL of TMB Substrate Solution to all wells.
13. Incubate the microwell strips at room temperature (18°C to 25°C) for 30 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.
14. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2°C to 8°C in the dark.
15. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IgG1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human IgG1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IgG1 concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:20,000 (predilution: 1:10,000; on the plate: 50 µL sample + 50 µL Assay Buffer (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 20,000).
- Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human IgG1 levels (Hook Effect). Such samples require further external predilution according to expected human IgG1 values with Assay Buffer (1x) in order to precisely quantitate the actual human IgG1 level.
- It is suggested that each testing facility establishes a control sample of known human IgG1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 7.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

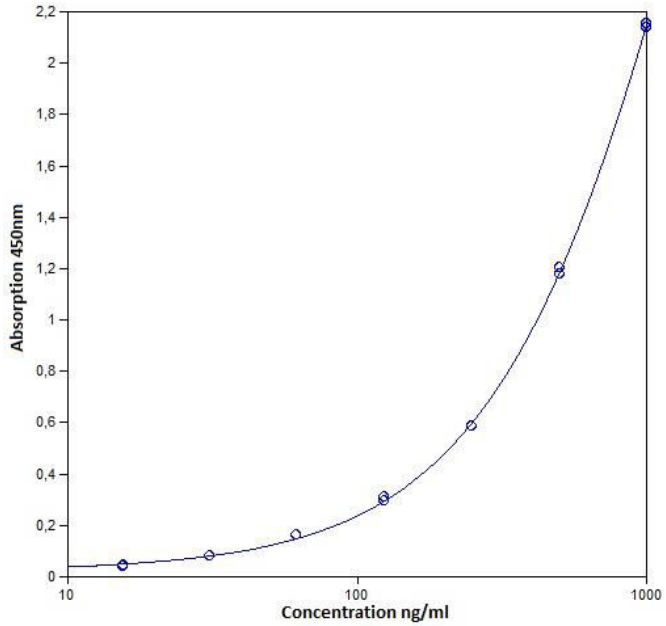

Fig. 7 Representative standard curve for human IgG1 ELISA. Human IgG1 was diluted in serial 2-fold steps in Assay Buffer (1x).

Table 2 Typical data using the human IgG1 ELISA.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	human IgG1 Concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	1000	2.177 2.162	2.169	0.3
2	500	1.199 1.226	1.212	1.1
3	250	0.608 0.606	0.607	0.2
4	125	0.331 0.316	0.324	2.3
5	62.5	0.185 0.184	0.184	0.3
6	31.3	0.101 0.105	0.103	1.9
7	15.6	0.062 0.065	0.063	2.6
Blank	0.0	0.025 0.027	0.026	4.3

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Performance characteristics

Sensitivity

The limit of detection of human IgG1 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.32 ng/mL (mean of 4 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 3 replicates of 8 serum and plasma samples, containing different concentrations of human IgG1. Two standard curves were run on each plate. Data below show the mean human IgG1 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 2.7%.

Table 3 The mean human IgG1 concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean human IgG1 concentration (ng/mL)	Coefficient of variation (%)
1	1	5222.4	2.3
	2	5385.0	2.3
	3	5405.1	1.2
2	1	3777.9	2.8
	2	4068.4	1.6
	3	4005.9	0.6
3	1	4005.8	2.7
	2	4350.1	0.9
	3	4118.1	2.5
4	1	4121.5	3.2
	2	4245.6	4.8
	3	4400.0	2.1
5	1	4130.7	2.5
	2	4579.9	1.2
	3	4488.8	4.2
6	1	8570.9	2.9
	2	9281.7	3.3
	3	9179.5	2.7
7	1	3930.4	5.0
	2	4153.7	3.3
	3	4203.8	4.0
8	1	4296.8	3.2
	2	4591.7	2.9
	3	4355.1	2.9

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum and plasma samples containing different concentrations of human IgG1. Two standard curves were run on each plate. Data below show the mean human IgG1 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 3.8%.

Table 4 The mean human IgG1 concentration and the coefficient of variation of each sample.

Sample	Mean human IgG1 concentration (ng/mL)	Coefficient of variation (%)
1	5337.5	1.9
2	3950.7	3.9
3	4158.0	4.2
4	4255.7	3.3
5	4399.8	5.4
6	9010.7	4.3
7	4096.0	3.6
8	4414.5	3.5

Dilution parallelism

Serum and plasma (EDTA, citrate, heparin) samples with different levels of human IgG1 were analyzed at serial 2-fold dilutions with 4 replicates each.

Sample matrix	Dilution	Recovery of Exp. Val. (%)	
		Mean	Range
Serum	1:40,000	102	94–110
	1:80,000	106	95–120
	1:160,000	85	73–99
Plasma (EDTA)	1:40,000	110	96–121
	1:80,000	115	95–136
	1:160,000	99	74–127
Plasma (citrate)	1:40,000	83	76–92
	1:80,000	81	75–85
	1:160,000	77	66–92
Plasma (heparin)	1:40,000	88	74–107
	1:80,000	89	74–121
	1:160,000	85	72–113

Sample stability

Freeze-thaw stability

Aliquots of serum and plasma samples (unspiked) were stored at -20°C and thawed 3 times, and the human IgG1 levels determined.

There was no significant loss of human IgG1 immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum and plasma samples (unspiked) were stored at –20°C, 2°C to 8°C, room temperature, and at 37°C, and the human IgG1 level determined after 24 hours. There was no significant loss of human IgG1 immunoreactivity detected during storage under above conditions.

Specificity

The assay detects both natural and recombinant human IgG1. The cross-reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IgG1 positive sample. No cross-reactivity or interference was detected.

Expected values

Panels of 20 serum as well as plasma samples (EDTA, citrate, heparin) from randomly selected donors (males and females) were tested for human IgG1.

Sample matrix	Number of samples evaluated	Mean (µg/mL)	Range (µg/mL)	Standard deviation (%)
Serum	20	5989	2355–11,514	41
Plasma (EDTA)	20	8349	3598–19,546	41
Plasma (citrate)	20	6152	2014–13,949	59
Plasma (heparin)	20	7175	2799–11,465	32

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 20x (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Assay buffer (1x)

Add Assay Buffer Concentrate 20x (5 mL) to 95 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

HRP-Conjugate

Make a 1:100 dilution of the concentrated HRP-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Human IgG1 standard

Reconstitute human IgG1 standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

Note: If instructions of this protocol have been followed, samples have been diluted 1:20,000 and the concentration read from the standard curve must be multiplied by the dilution factor (x20,000).

1. Predilute your samples with Assay Buffer (1x) 1:10,000.
2. Determine the number of microwell strips required.
3. Prepare HRP-Conjugate.
4. Wash microwell strips twice with Wash Buffer.
5. Standard dilution on the microwell plate: Add 100 µL Assay Buffer (1x), in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

   Alternatively, external standard dilution in tubes (see "External standard dilution" on page 3): Pipette 100 µL of these standard dilutions in the microwell strips.
6. Add 100 µL of Assay Buffer (1x) in duplicate to the blank wells.
7. Add 50 µL of Assay Buffer (1x) to the sample wells.
8. Add 50 µL of each sample in duplicate to the sample wells.
9. Add 50 µL diluted HRP-Conjugate to all wells.
10. Cover microwell strips and incubate 1 hours at room temperature (18°C to 25°C) on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance).
11. Empty and wash microwell strips 4 times with Wash Buffer.
12. Add 100 µL of TMB Substrate Solution to all wells.
13. Incubate the microwell strips for about 30 minutes at room temperature (18°C to 25°C)
14. Add 100 µL Stop Solution to all wells.
15. Blank microwell reader and measure color intensity at 450 nm.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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