



AssayMax™
Human IgG ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 μ l of Standard or Sample per well.

Incubate 2 hours.

Step 2. Wash, then add 50 μ l of Biotinylated Antibody per well.

Incubate 1 hour.

Step 3. Wash, then add 50 μ l of SP Conjugate per well.

Incubate 30 minutes.

Step 4. Wash, then add 50 μ l of Chromogen Substrate per well.

Incubate in ambient **LIGHT** for 15 minutes.

Step 5. Add 50 μ l of Stop Solution per well.

Read at 450 nm immediately.

Symbol Key



Consult instructions for use.

Assay Template

[illegible]

AssayMax™ Human Immunoglobulin G (IgG) ELISA Kit

Catalog No. EI7200-1

Sample insert for reference use only

Introduction

Human immunoglobulin G (IgG), the most abundant antibody in serum, constitutes 75% of serum immunoglobulins. IgG is synthesized and secreted by plasma B cells and contains two heavy chains and two light chains. IgG has four subclasses IgG1, IgG2, IgG3, and IgG4 and is involved in the secondary immune response. As it is the only isotype that can pass through the human placenta, maternal IgG provides the defense against infection for the first few weeks of a neonate (1). IgG has been shown to treat autoimmune disease, induce apoptosis, and stimulate complement attenuation (2-4). Elevated IgG is observed in viral hepatitis, autoimmune hepatitis, and cirrhosis (5).

Principle of the Assay

The AssayMax™ Human Immunoglobulin G ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of IgG in human **plasma, serum, milk, urine, saliva, CSF, cell culture supernatant, cell lysate, and tissue samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures human IgG in approximately 4 hours. A polyclonal antibody specific for human IgG has been pre-coated onto a 96-well microplate with removable strips. IgG in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human IgG, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate), as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.

- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human IgG Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human IgG.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human IgG Standard:** Human IgG in a buffered protein base, calibrated against NIBSC 67/099 (64 ng, lyophilized).
- **Biotinylated Human IgG Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against human IgG (120 µl).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **SP Conjugate (100x):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate (1x):** A stabilized peroxidase chromogen substrate tetramethylbenzidine (7 ml).
- **Stop Solution (1x):** A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction (11 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant (EDTA or Heparin can also be used as an anticoagulant). Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma. A 2000000-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. A 2000000-fold sample dilution is suggested into EIA Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. A 2000-fold sample dilution is suggested into EIA Diluent or within the range of 500x – 10000x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. A 100-fold sample dilution is suggested into EIA Diluent or within the range of 10x – 4000x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. A 1000-fold sample dilution is suggested into EIA Diluent or within the range of 100x – 10000x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. A 4000-fold sample dilution is suggested into EIA Diluent or within the range of 400x – 40000x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatant:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into EIA Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

- **Cell Lysate:** Rinse cell with cold phosphate-buffered saline (PBS) and then scrape the cell into a tube that contains 5 ml of cold PBS with 5 mM EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1×10^6 cells, add approximately 100 μ l of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. If necessary, dilute samples into EIA Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with PBS containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into EIA Diluent; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater <i>(for reference only; please follow the insert for specific dilution suggested)</i>	
100x	10000x
A) 4 μ l sample : 396 μ l buffer (100x) = 100-fold dilution <i>Assuming the needed volume is less than or equal to 400 μl.</i>	A) 4 μ l sample : 396 μ l buffer (100x) B) 4 μ l of A : 396 μ l buffer (100x) = 10000-fold dilution <i>Assuming the needed volume is less than or equal to 400 μl.</i>
1000x	100000x
A) 4 μ l sample : 396 μ l buffer (100x) B) 24 μ l of A : 216 μ l buffer (10x) = 1000-fold dilution <i>Assuming the needed volume is less than or equal to 240 μl.</i>	A) 4 μ l sample : 396 μ l buffer (100x) B) 4 μ l of A : 396 μ l buffer (100x) C) 24 μ l of B : 216 μ l buffer (10x) = 100000-fold dilution <i>Assuming the needed volume is less than or equal to 240 μl.</i>

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.

- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
- **Human IgG Standard:** Reconstitute the Human IgG Standard (64 ng, 0.8 mIU) with 1.6 ml of EIA Diluent to generate a 40 ng/ml (0.5 mIU/ml) standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (40 ng/ml) 2-fold with equal volume of EIA Diluent to produce 20, 10, 5, 2.5, 1.25, and 0.625 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[IgG] (ng/ml)	[IgG] (mIU/ml)
P1	1 part Standard	40	0.5
P2	1 part P1 + 1 part EIA Diluent	20	0.25
P3	1 part P2 + 1 part EIA Diluent	10	0.125
P4	1 part P3 + 1 part EIA Diluent	5.0	0.063
P5	1 part P4 + 1 part EIA Diluent	2.5	0.031
P6	1 part P5 + 1 part EIA Diluent	1.25	0.016
P7	1 part P6 + 1 part EIA Diluent	0.625	0.008
P8	EIA Diluent	0.0	0.0

- **Biotinylated Human IgG Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with EIA Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with EIA Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).

- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μ l of Human IgG Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 μ l of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 μ l of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μ l of Biotinylated Human IgG Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μ l of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μ l of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 15 minutes or until the optimal blue color density develops.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical Data

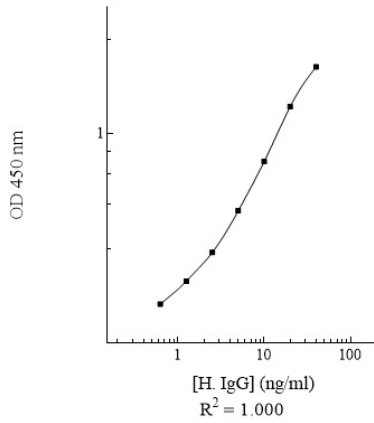
- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	40	2.088 2.062	2.075
P2	20	1.308 1.372	1.340
P3	10	0.718 0.750	0.734
P4	5.0	0.413 0.441	0.427
P5	2.5	0.261 0.279	0.270
P6	1.25	0.190 0.204	0.197
P7	0.625	0.148 0.158	0.153
P8	0.0	0.108 0.102	0.105
Sample: Pooled Normal Sodium Citrate Plasma (2000000x)		0.408 0.446	0.427
Sample: Pooled Normal Serum (2000000x)		0.649 0.599	0.624

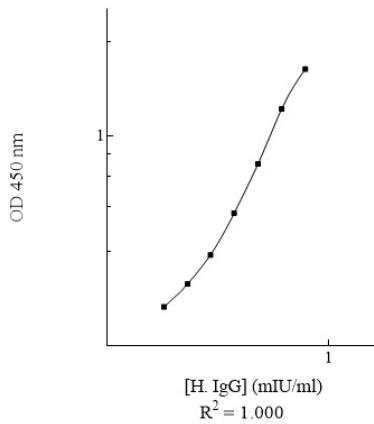
Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Human IgG Standard Curve



Human IgG Standard Curve



Reference Value

- Normal human IgG plasma and serum levels range from 6 – 18 mg/ml.
- Plasma and serum samples from healthy adults were tested (n=20). On average, human IgG level was 13.5 mg/ml.

Sample	n	Average Value (mg/ml)
Pooled Normal Plasma	10	10.3
Pooled Normal Serum	10	16.6

Performance Characteristics

- **Kit standard has been calibrated against NIBSC 67/099.**
- The minimum detectable dose of human IgG as calculated by 2SD from the mean of a zero standard was established to be 0.43 ng/ml.
- Intra-assay precision was determined by testing three plasma samples ten times in one assay.
- Inter-assay precision was determined by testing three plasma samples in ten assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	10	10	10
CV (%)	6.0%	6.1%	6.5%	9.4%	7.4%	8.5%
Average CV (%)	6.2%			8.4%		

Spiking Recovery

- Recovery was determined by spiking one plasma and one serum sample with different human IgG concentrations.

Sample	Unspiked Sample (ng/ml)	Spiking Value (ng/ml)	Expected	Observed	Recovery (%)
Plasma	9.273	4.602	13.875	12.408	89%
		2.324	11.597	11.680	101%
		1.216	10.489	10.295	98%
Serum	9.725	4.602	14.327	15.350	107%
		2.324	12.049	11.686	97%
		1.216	10.941	10.658	97%
Average Recovery (%)					98%

Linearity

- Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1000000x	94%	104%
2000000x	91%	99%
4000000x	110%	98%

Cross-Reactivity

Species	Cross-Reactivity (%)
Canine	None
Bovine	None
Equine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None
Protein	Cross-Reactivity (%)
Human IgG1	< 50%
Human IgG2	< 5%
Human IgG3	< 30%
Human IgG4	< 50%
Human IgM	< 5%

- No significant cross-reactivity observed with human IgA, IgA1, IgA2, IgD, IgE, and IgJ proteins.
- 10% FBS in culture media will not affect the assay.

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.

Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	- Check pipette calibration. - Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
Deficient Standard Curve Fit	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.
	Non-optimal sample dilution	- Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. - Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. - User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	- Pipette properly in a controlled and careful manner. - Check pipette calibration. - Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	- Thoroughly agitate the lyophilized components after reconstitution. - Thoroughly mix dilutions.

References

- (1) Pitcher-Wilmott RW *et al.* (1980) *Clin Exp Immunol.* 41(2):303-308.
- (2) Clancy RM *et al.* (2004) *Arthritis Rheum.* 50(1):173-182.
- (3) Eray M *et al.* (1994) *Int Immunol.* 6(12):1817-1827.
- (4) Lutz HU *et al.* (2004) *Blood.* 103(2):465-472.
- (5) Teufel A *et al.* (2009) *World J Gastroenterol.* 15(9):1035-1041.

Version 3.2