



Human IgG ELISA Kit

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Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

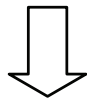
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

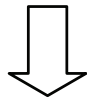
Thank you for choosing Assaypro.

Assay Summary

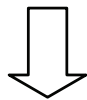
Add 50 μ l of Standard/ Sample per well.
Incubate 2 hours.



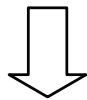
Wash, then add 50 μ l of
Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add 50 μ l of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 μ l of
Chromogen Substrate per well.
Incubate 12 minutes.



Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

AssayMax Human IgG ELISA Kit

Catalog No. EI7200-1
Sample Insert/Reference 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 IgG ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of human IgG in plasma, serum, saliva, urine, milk, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human IgG in less than 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 the biotinylated polyclonal antibody specific for IgG, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then 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

- **Prepare all reagents (working diluent buffer, wash buffer, standards, 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 protocol. 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.**
- This kit is for research use only.

- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

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 (160 ng, lyophilized).
- **Biotinylated Human IgG Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against IgG (140 μ l).
- **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).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 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.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- 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 μ l, 20-200 μ l, 200-1000 μ l, 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. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:600000 into EIA Diluent or within the range of 1:100000 to 1:1000000, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant.)
- **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. Dilute samples 1:600000 into EIA Diluent or within the range of 1:100000 to 1:1000000, and assay. 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. Dilute urine 1:100 into EIA Diluent and assay. 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. Dilute saliva 1:1000 into EIA Diluent and assay. 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. Dilute milk 1:2000 into EIA Diluent and assay. 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. Dilute samples 1:4000 into EIA Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 160 ng (2.8 mU) of Human IgG Standard with 2 ml of EIA Diluent to generate an 80 ng/ml (1.4 mU/ml) standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate

standard points by serially diluting the standard solution (80 ng/ml) 1:2 with EIA Diluent to produce 40, 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 solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[IgG] (ng/ml)	[IgG] (mU/ml)
P1	1 part Standard (80 ng/ml) + 1 part EIA Diluent	40.00	0.700
P2	1 part P1 + 1 part EIA Diluent	20.00	0.350
P3	1 part P2 + 1 part EIA Diluent	10.00	0.175
P4	1 part P3 + 1 part EIA Diluent	5.000	0.088
P5	1 part P4 + 1 part EIA Diluent	2.500	0.044
P6	1 part P5 + 1 part EIA Diluent	1.250	0.022
P7	1 part P6 + 1 part EIA Diluent	0.625	0.011
P8	EIA Diluent	0.000	0.000

- **Biotinylated Human IgG Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen 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 µl of Human IgG Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

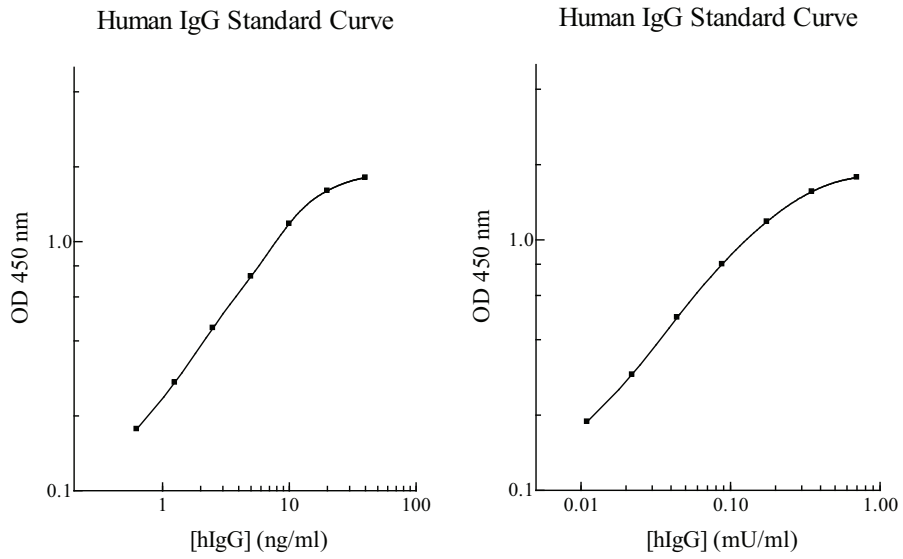
- Add 50 μ l of Biotinylated Human IgG Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μ l of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μ l of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.
- 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 on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

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



Sensitivity and Specificity

- The minimum detectable dose of IgG is typically ~ 0.6 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.2% respectively.
- **Kit Standard has been calibrated against WHO International Standard.**

Linearity

Sample Dilution	Plasma	Serum
1:300000	89%	92%
1:600000	96%	97%
1:1200000	106%	106%

Recovery

Standard Added Value	1.25 – 20 ng/ml
Recovery %	84 – 116%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None
Immunoglobulins	% Cross Reactivity
IgA	None
IgA1	None
IgA2	None
IgD	None
IgE	None
IgG1	<50%
IgG2	<5%
IgG3	<30%
IgG4	<50%
IgM	<20%

Reference Value

- Normal human IgG plasma levels range from 6 to 18 mg/ml.

References

- (1) Pitcher-Wilmott RW *et al.* (1980) *Clin. Exp. Immunol.* 41, 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* 15:103(2):465-472
- (5) Teufel A *et al.* (2009) *World J Gastroenterol.* 15(9):1035-1041

Version 1.5