Human IgG3 ELISA Kit

Introduction

Human Immunoglobulin G (IgG), the most abundant antibody in the 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 attenuate complement (3-4). Elevated IgG is observed in viral hepatitis, autoimmune hepatitis and cirrhosis (5).

Principal of the Assay

The Human IgG3 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human IgG3 in plasma, serum, urine, saliva, milk, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human IgG3 in less than 4 hours. A polyclonal antibody specific for human IgG3 has been pre-coated onto a 96-well microplate with removable strips. IgG3 in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for IgG3, 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 acid solution

Reagents

- **Human IgG3 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human IgG3.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.

- **Human IgG3 Standard:** Human IgG3 in a buffered protein base (50 ng, lyophilized).
- **Biotinylated IgG3 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against IgG3 (140 µl).
- **MIX 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 µl).
- **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

- Store components of the kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20^{0}C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8^{0}C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8^{0}C.
- Store Standard at 2-8^{0}C before reconstituting with Diluent and at -20^{0}C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000µl 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 2000 x g for 10 minutes and assay. Dilute samples 1:100000 into MIX Diluent. If necessary dilute samples within the range of 1:50000 to 1:200000. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:100000 into MIX Diluent. If necessary dilute samples within the range of 1:50000 to 1:200000. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes. Dilute Urine 1:4 with MIX Diluent. If necessary dilute samples within the range of 1:2 to 1:8. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes. Dilute 1:40 with MIX Diluent. If necessary dilute samples within the range of 1:20 to 1:80. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes. Dilute Milk 1:500 with MIX Diluent. If necessary dilute samples within the range of 1:250 to

1:1000. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 50 ng of IgG3 Standard with 2 ml of MIX Diluent to generate a solution of 25 ng/ml. 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 (25 ng/ml) 1:2 with MIX Diluent to produce 12.5, 6.25, 3.13, 1.56, 0.781, and 0.391 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[IgG3] (ng/ml)
P1	Standard Stock (25 ng/ml)	25.0
P2	1 part P1 + 1 part MIX Diluent	12.5
P3	1 part P2 + 1 part MIX Diluent	6.25
P4	1 part P3 + 1 part MIX Diluent	3.13
P5	1 part P4 + 1 part MIX Diluent	1.56
P6	1 part P5 + 1 part MIX Diluent	0.781
P7	1 part P6 + 1 part MIX Diluent	0.391
P8	MIX Diluent	0.000

- **Biotin IgG3 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20^{0}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 MIX Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of IgG3 standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated IgG3 Antibody to each well and incubate for one hour.
- Wash the microplate as described above.
- Add 50 µl 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.

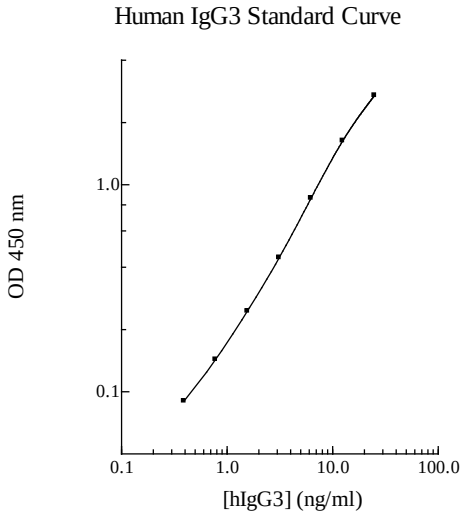
- Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- 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 IgG3 is typically ~ 0.3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.2% and 7.4% respectively.

Linearity

Sample Dilution	Plasma	Serum
1:50000	91%	92%
1:100000	96%	97%
1:200000	102%	101%

Sample Dilution	Saliva
1:250	86%
1:1000	95%
1:2000	109%

Sample Dilution	Urine
1:2	86%
1:4	98%
1:8	110%

Sample Dilution	Milk
1:250	92%
1:500	97%
1:1000	101%

Recovery

Standard Added Value	0.1 – 1 ng/ml
Recovery %	87 - 106 %
Average Recovery %	97.5%

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	1%
IgG2	0.5%
IgG3	100%
IgG4	0.5%
IgM	None