Human GC-Globulin ELISA Kit

Introduction

GC-Globulin or Vitamin D-binding protein is a multifunctional plasma protein with functions in the transport of vitamin D metabolites, control of bone development, binding of fatty acids, sequestration of actin and a range of less-defined roles in modulating immune and inflammatory responses (1). The GC-Globulin levels on healthy individuals range from 176-623mg/L with no age dependency (2). A low serum level (< 100 mg/L) of the actin-scavenger GC-globulin is a prognostic marker of non-survival in fulminant hepatic failure (FHF) (3), trauma and sepsis (4). Low GC-globulin plasma or serum levels are also linked to osteoporosis, Graves' disease, Hashimoto's thyroiditis, diabetes, COPD, AIDS, multiple sclerosis, sarcoidosis and rheumatic fever (5).

Principal of the Assay

The Human GC-Globulin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human GC-Globulin in plasma, serum and cell culture supernatants. This assay employs a quantitative competitive enzyme immunoassay technique that measures human GC-Globulin in less than 3 hours. A polyclonal antibody specific for human GC-Globulin has been pre-coated onto a 96-well microplate with removable strips. GC-Globulin in standards and samples is competed with a biotinylated GC-Globulin sandwiched by the immobilized antibody and 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-protein, 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 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 GC-Globulin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human GC-Globulin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Human GC-Globulin Standard:** Human GC-Globulin in a buffered protein base (100 µg, lyophilized).
- **Biotinylated GC-Globulin:** 1 vial, lyophilized.
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **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.
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 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. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:400 in EIA Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (Heparin and EDTA can also be used as an 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 1:400 into EIA Diluent. Store serum at -20^{0}C or below. 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 samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 100 µg of GC-Globulin Standard with 1 ml of EIA Diluent to generate a solution of 100 µg/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 (100 µg/ml) 1:4 with EIA Diluent to generate 25, 6.25, 1.56, 0.391 and 0.098 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[hGC-Globulin] (µg/ml)
P1	Standard (100 µg/ml)	100.000
P2	1 part P1 + 3 parts EIA Diluent	25.000
P3	1 part P2 + 3 parts EIA Diluent	6.250
P4	1 part P3 + 3 parts EIA Diluent	1.563
P5	1 part P4 + 3 parts EIA Diluent	0.391
P6	1 part P5 + 3 parts EIA Diluent	0.098
P7	EIA Diluent	0.000

- **Biotinylated GC-Globulin (1x):** Dilute Biotinylated GC-Globulin with 4 ml EIA Diluent to use. Allow to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 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^{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 25 µl of standard or sample per well, and immediately add 25 µl of Biotinylated GC-Globulin to each well (on top of the Standard or sample) and mix gently. 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 Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 8 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.

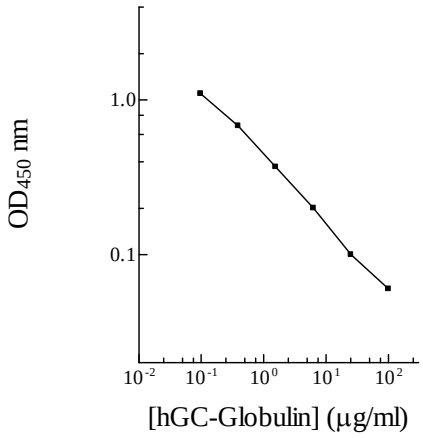
Data Analysis

- 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 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.

Standard Curve

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

H. GC-Globulin
Standard Curve



Performance Characteristics

- The minimum detectable dose of GC-Globulin is typically ~0.09 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.5% respectively.
- No significant cross-reactivity or interference was observed.

References

(1) Gomme PT *et al.* (2004) *Trends Biotechnol.* 22(7): 340-5
(2) Jorgensen CS *et al.* (2204) *Scand J Clin Lab Invest.* 64(2): 157-66
(3) Schiodt FV *et al.* (2001) *Scand J Gastroenterol.* 36(9): 998-1003
(4) Dahl B *et al.* (2003) *Crit Care Med.* Jan; 31(1): 152-6
(5) Speeckaert M *et al.* (2006) *Clin Chim Acta.* 372(1-2): 33-42. Epub 2006 May 12

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