KRIBIOLISA™ Human IgG ELISA

REF: KBBP08

Ver 2.5

RUO

Immunoassay for the Quantification of Human IgG in serum, plasma and cell culture supernatant

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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KRIBIOLISA™ Human IgG ELISA

Introduction:

Human IgG is a monomeric immunoglobulin, and the most abundant one constituting approximately 75% of the serum. Presence of such trace contaminants in the products may lead to potential health hazards, especially when the product is intended for therapeutic use.

Intended Use:

This generic kit is intended in determining the presence of Total human Immunoglobulin G contamination in variety of sample types. A highly sensitive and specific Enzyme linked immunosorbent assay can be used for the quantitation of low levels of human IgG.

Materials Provided:

1. Anti-human IgG Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Human IgG Standards, (conc. 50 ug/ml, lyophilized) - 2 vials
3. Human IgG Biotin Conjugated Detection antibody - 1 vial
4. Streptavidin HRP Conjugate (conc.) - 1 vial
5. (1X) Assay Diluent - 50 ml
6. Streptavidin HRP Diluent -12 ml
7. (20X) Wash Buffer - 25 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettes to measure volumes ranging from 25 ul to 1000 ul.
3. Distilled water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent paper.

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solution are stable until the expiration date of the kit.
3. Before using, bring all components to Room Temperature (18-25ºC). Upon assay completion return all components to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. All the reagents provided may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing use only.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation (all reagents should be diluted immediately prior to use):
Please refer to lot specific Certificate of analysis or preparation of reagents

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
2. If the IgG concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. High Dose Hook Effect may be observed in samples with very high concentrations of hIgG, usually in samples from the initials stages of purifications. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of samples containing sodium azide (NaN_3), as it may destroy the HRP activity of the conjugate resulting in the under-estimation of the levels of hIgG.
4. All Standards and Samples should be assayed at least in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicates. A standard curve is required for each assay.

2. **Standards Preparation:** Reconstitute the lyophilized vial with 20 ul of Distilled water to generate a 50 ug/ml concentration. Keep the standard for 15 mins with gentle agitation before making further dilutions. Dilute 2 ul of original Standard (50 ug/ml) with 98 ul of Assay diluent (1X) to generate a 1 ug/ml Standard solution. Perform serial dilutions by using main stock solution as per the below table. Assay Diluent (1X) serves as the zero standard (0 ng/ml).

Standard Concentration	Standard No	Dilution Particulars
50 ug/ml (Lyophilized)	Standard Main stock	Original Standard provided in the Kit + Reconstitute in 20 ul Distilled Water
1 ug/ml	Mid Stock	2 ul of Original Standard + 98 ul Assay Diluent (1X)
10 ng/ml	Standard No.7	10 ul Middle Stock + 990 ul Assay Diluent (1X)
8 ng/ml	Standard No.6	800 ul Standard No.7 + 200 ul Assay Diluent (1X)
6 ng/ml	Standard No.5	750 ul Standard No.6 + 250 ul Assay Diluent (1X)
4 ng/ml	Standard No.4	666.67 ul Standard No.5 + 333.33 ul Assay Diluent (1X)
3 ng/ml	Standard No.3	750 ul Standard No.4 + 250 ul Assay Diluent (1X)
2 ng/ml	Standard No.2	666.67 ul Standard No.3 + 333.33 ul Assay Diluent (1X)
1 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent (1X)

3. Add **100 ul of prepared standards** and **samples** into the respective wells and cover the plate and incubate it for 2 hour at room temperature.

4. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

5. Add **100 ul** of **biotinylated hIgG antibody** into each well.

6. Cover the plate and incubate it for 1 hour at room temperature.

7. Aspirate and wash plate **4 times** and with **Wash Buffer (1X)** as mentioned in the Step No 4.

8. Add **100 ul** of **Streptavidin HRP Conjugate** into each well.

9. Cover the plate and incubate it for 1 hour at room temperature.

10. Aspirate and wash plate **4 times** and with **Wash Buffer (1X)** as mentioned in the Step No 4.

11. Pipette out **100 ul** of **TMB Substrate** in each well.

12. Incubate the plate at room temperature for 15 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

13. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

14. Read the absorbance at 450 with a microplate reader.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points.

To determine the unknown Human IgG concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the concentration. If samples were diluted, multiply by the appropriate dilution factor.

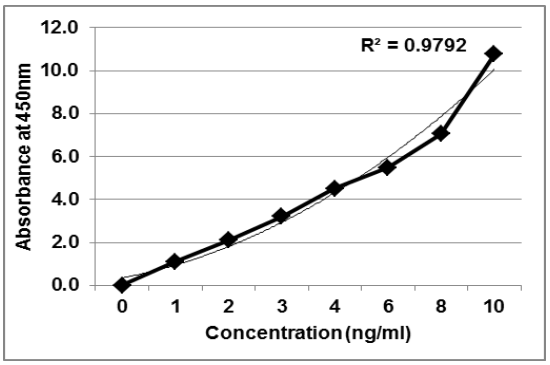
Software which is able to generate a polynomial regression (2nd order) / 4-PL or a cubic spline curve-fit is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Typical Data

Standards (ng/ml)	Abs 1	Abs 2	Mean Abs	Interpolated Concentration	% Interpolated Concentration
0	0.094	0.092	0.093	--	--
1	0.479	0.457	0.468	1.113	111.3
2	0.747	0.736	0.742	2.110	105.5
3	1.036	1.055	1.046	3.215	107.2
4	1.538	1.274	1.406	4.521	113.0
6	1.646	1.693	1.669	5.475	91.3
8	2.098	2.115	2.106	7.059	88.2
10	3.065	3.191	3.128	10.761	107.6

Typical Graph



Performance Characteristics:

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to 0.5 ng/ml

Specificity / Cross reactivity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Human IgG.

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Zero Std Zero Std			
1B 2B	1 ng/ml 1 ng/ml			
1C 2C	2 ng/ml 2 ng/ml			
1D 2D	3 ng/ml 3 ng/ml			
1E 2E	4 ng/ml 4 ng/ml			
1F 2F	6 ng/ml 6 ng/ml			
1G 2G	8 ng/ml 8 ng/ml			
1H 2H	10 ng/ml 10 ng/ml			
3A 4A	Sample			
3B 4B	Sample			

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