KRIBIOLISA® Mouse IgG ELISA

REF : KBI1003

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Mouse IgG in serum, plasma and other biological samples

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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REF KBI1003 Σ 96 tests

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Introduction:

A variety of antibodies are used as therapeutics agent as well as in pharmaceuticals. These antibodies are generally purified through protein A column chromatography. This method of purification has the potential with contamination of protein A, which might get leached out of the column and co-eluted with purified antibody. This may result in adverse toxic or immunological reactions that ultimately affect the efficacy of the product antibody. The highly sensitive Mouse IgG ELISA is a quantitative, simple and will aid in monitoring the purification process as well as screening product prior to its use.

Intended Use:

The KRIBIOLISA® Mouse IgG ELISA kit is used as an analytical tool for quantitative determination of Mouse IgG in serum, plasma and other biological samples.

Principle:

The method employs Capture ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and Standards are pipetted into microwells and Anti-Mouse IgG present in the sample are bound by the antibodies. Anti-Mouse IgG Conjugated to HRP Antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Mouse IgG in the sample. The color development is stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-Mouse IgG Antibody coated Microtiter plate (12 x 8 wells) - 1 no
2. Mouse IgG Standards, (0, 0.02, 0.1, 0.2, 0.75 and 1.9 ug/ml) – 6 x 0.3 ml
3. Anti-Mouse IgG Antibody:HRP Conjugate - 12 ml
4. Sample Diluent - 30 ml
5. Detection Antibody - 12 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12 ml
8. 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 pipette or to measure volumes ranging from 25 ul to 1000 ul.
3. Deionised (DI) 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 solutions 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. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing Use only.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room Temperature before use.

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. High Dose Hook Effect may be observed in samples with very high concentrations of Mouse IgG. High Dose Hook Effect is due to insufficient excess of antibody for very high concentrations of Mouse IgG present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Mouse IgG concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Mouse IgG.
4. All Standards, Controls 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.

Sample Treatment Procedure

Dilute the samples in the sample diluent to obtain a concentration between 0.006 ug/ml and 2 ug/ml.

Samples	Recommended Dilutions
Cell Culture Supernatant	1/100
Miniperm®, KRIBIOLISA®	1/1000
Ascitic Fluid/sera	1/5000

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. Pipette out **20 ul** of **Standards** and **Samples** into the respective wells.

3. Pipette out **100 ul** of **Anti-Mouse IgG Antibody:HRP Conjugate** into each well.

4. Cover the plate and incubate at RT for 15 minutes.

5. Aspirate and wash plate 3 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.

6. Add **100 ul** of **TMB Substrate** in each well.

7. Incubate the plate at room temperature for 10 minutes in dark. DO NOT SHAKE or else it may affect precision.

8. Pipette out **100 ul** of **Stop Solution**.

9. Read the absorbance at 450 nm.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) 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 Mouse 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 Mouse IgG Concentration. If samples were diluted, multiply by the appropriate dilution factor.

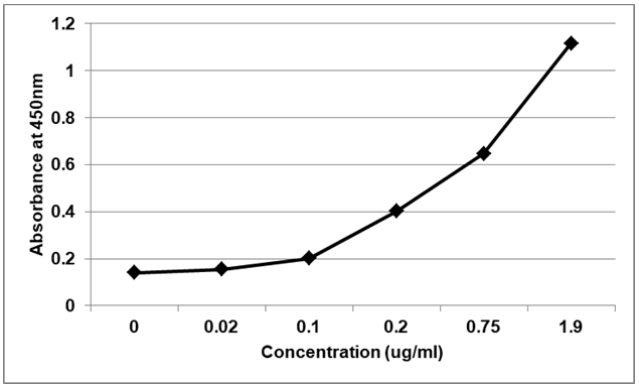
Software which is able to generate a cubic spline curve-fit or a polynomial curve (2^{nd} order) is best recommended for automated results.

Note: To estimate the rate of contamination of an antibody with Mouse IgG, the concentration of Mouse IgG obtained may be reported (in ppm) to the concentration of the antibody. Example: the concentration of protein A is detected at 800 ug/ml in an antibody with a concentration of 1 mg/ml, therefore the antibody is contaminated with 0.8 ppm Mouse IgG.

Typical Data

Standard Concentration (ug/ml)	Mean Abs
0	0.141
0.02	0.154
0.1	0.202
0.2	0.401
0.75	0.648
1.9	1.117

Typical Graph



Abs = absorbance

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

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.

Performance Characteristics of the Kit:
Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature.

We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the

protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 0.01 ug/ml.

Specificity:
The antibodies used in the kit for capture and detection are monoclonal antibodies specific for Mouse IgG. No cross reactivity was observed with samples containing IgG antibodies.

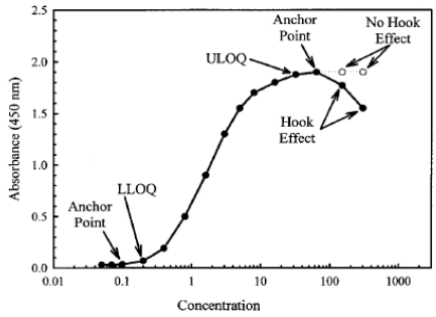
Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Mouse IgG and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
(n=5)	84-107%	87-108%	82-112%

Standard Calibration Range:
63 ug/ml – 4000 ug/ml

High Dose Hook Effect:
The high dose hook effect refers to measured levels of antigen displaying a significantly lower absorbance than the actual level present in a sample. This appears when a simultaneous ELISA assay is saturated by a very high concentration of sample antigen binding to all available sites on both the solid phase antibody as well as the detection antibody and thereby preventing the sandwich-formation. The antigen-saturated detection antibodies in solution will be washed off giving a falsely low signal. A “hook” is observed in the curve when data is plotted as a signal versus antigen concentration.



A high dose hook is indicated in the plotted curve when the assay is saturated by high antigen concentrations.

Increasing concentrations of Mouse IgG >5 ug/ml were assayed as unknowns.

Safety Precautions:

- **This kit is For Research and Manufacturing Use Only**. 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.
- 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.

Example of a Work List

Well #	Contents	Abs at 450	Mean Absorbance	ug/ml Mouse IgG concentration
1A 2A	Zero Std. Zero Std.			
1B 2B	0.02 ug/ml 0.02 ug/ml			
1C 2C	0.1 ug/ml 0.1 ug/ml			
1D 2D	0.2 ug/ml 0.2 ug/ml			
1E 2E	0.75 ug/ml 0.75 ug/ml			
1F 2F	1.9 ug/ml 1.9 ug/ml			
1G 2G	Sample Sample			
1H 2H	Sample Sample			

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