

KRIBIOLISA™ Human Immunoglobulin A (IgA) ELISA

REF: KBBP09

Ver 4.2

RUO

Immunoassay for quantitative determination of Human IgA in cell culture supernatant and other biological preparations

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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 96 tests



KRISHGEN BioSystems

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Introduction:

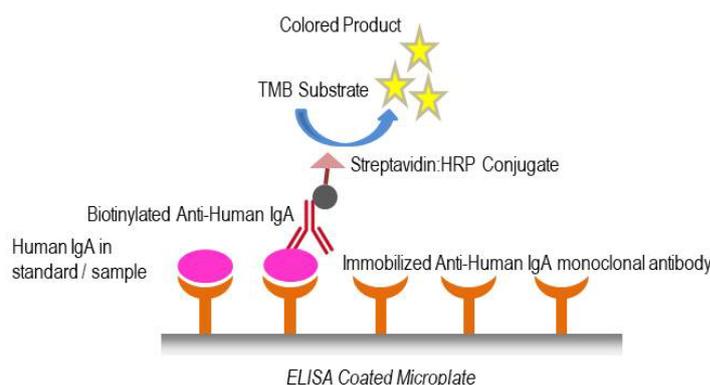
Human IgA is an antibody playing a critical role in mucosal immunity, and found in the form of secretory IgA polymer in the secretions. Presence of such a trace contaminants in the products may lead to potential health hazards, especially when the product is intended for therapeutic use.

Intended Use:

This generic kit is intended in determining the presence of human immunoglobulin A in variety of sample types - cell culture supernatant and other biological preparations. This highly sensitive and specific Enzyme linked immunosorbent assay can be used for the quantitation of low level of human IgA. The kit is not intended for use on human serum and plasma samples.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing IgA react with already coated affinity purified capture anti-Human IgA antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Biotinylated Anti-Human IgA is added leading to formation of a sandwich complex of solid phase antibody-Human IgA-enzyme labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. Streptavidin:HRP conjugate is added which binds to Biotinylated Anti-Human IgA complex. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3, 3',5, 5' Tetra Methyl Benzidine is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of Human IgA present in the samples.



PRINCIPLE OF THE KRIBIOLISA™ Human IgA ELISA

Materials Provided:

Part	Description	Qty
Anti-Human IgA Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Human IgA monoclonal antibody.	1 x 96 wells
Recombinant Human IgA Standard	Recombinant Human IgA Standard, lyophilized (370 ug/ml)	2 vials
Biotinylated Anti-Human IgA	Biotinylated Anti-Human IgA antibody, concentrated	1 vial
Streptavidin:HRP Conjugate	Streptavidin:HRP Conjugated, concentrated	1 vial
Streptavidin HRP Diluent	Buffered protein base with preservative thiomersol <0.01% with 3% BSA	12 ml
(1X) Assay Diluent	Buffered protein base with preservative thiomersol <0.01%	50 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	0.73M Phosphoric Acid	12 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at 2-8°C.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. After reconstitution of standards, it has to be used immediately and cannot be stored.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

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 < -20°C. Avoid repeated freeze/thaw cycles.

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. If the Human IgA 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 Human IgA, 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 (NaN₃), as it may destroy the HRP activity of the conjugate resulting in the under-estimation of the levels of Human IgA.
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.

Preparation before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity.

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.
3. To make **(1X) Wash Buffer**; dilute **25 ml of (20X) Wash Buffer** in **475 ml of DI water**.
4. **Biotinylated Anti-Human IgA (concentrated)**: prepare the **biotinylated Anti-Human IgA working solution** as per the attached sheet available with the kit. Please note as the concentrations offered vary from lot to lot, do not mix the components of different lots when running the assay.
5. **Streptavidin:HRP Conjugate (concentrated)**: prepare the **Streptavidin:HRP Conjugate working solution** as per the attached sheet available with the kit. Please note as the concentrations offered vary from lot to lot, do not mix the components of different lots when running the assay.
6. Reconstitute the **lyophilized standard** in 20 ul of distilled water to get concentration of 370 ug/ml. Add 1 ul of reconstituted standard to 369 ul of Assay Diluent (1X) to get concentration of 1 ug/ml. Do further dilutions of the standards Thus, the Human IgA standard concentrations are 200 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml and 3.125 ng/ml. **Assay Diluent (1X)** serves as the zero standard (0 ng/ml).

Standard Concentration	Standard Vial	Dilution Particulars
370 ug/ml	Original Standard	Original Standard provided in the Kit + 20 ul of Distilled water
1 ug/ml	Middle Stock	1 ul of Original Standard + 369 ul of Assay Diluent (1X)
200 ng/ml	Standard No.7	100 ul Middle Stock (1 ug/ml) + 400 ul Assay Diluent (1X)
100 ng/ml	Standard No.6	250 ul Standard No.7 + 250 ul Assay Diluent (1X)
50 ng/ml	Standard No.5	250 ul Standard No.6 + 250 ul Assay Diluent (1X)
25 ng/ml	Standard No.4	250 ul Standard No.5 + 250 ul Assay Diluent (1X)
12.5 ng/ml	Standard No.3	250 ul Standard No.4 + 250 ul Assay Diluent (1X)
6.25 ng/ml	Standard No.2	250 ul Standard No.3 + 250 ul Assay Diluent (1X)
3.125 ng/ml	Standard No.1	250 ul Standard No.2 + 250 ul Assay Diluent (1X)
0 ng/ml	Standard No.0	Only Assay Diluent (1X)

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human IgA. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human IgA 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 Human IgA 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 (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of Human IgA.
4. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
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 duplicate or triplicate. A standard curve is required for each assay.
2. Pipette **100 ul** of **prepared Standards** or **Samples** into the respective wells.
3. Cover the plate and incubate for 120 minutes 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.
5. Add **100 ul** of **Biotinylated Anti-Human IgA antibody working solution** into each well.
6. Cover the plate and incubate for 60 minutes at Room Temperature.
7. Add **100 ul** of **Streptavidin:HRP Conjugate working solution** into each well.
8. Cover the plate and incubate for 60 minutes at Room Temperature.
9. 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.
10. Add **100 ul** of **TMB Substrate** in each well.
11. Incubate the plate at Room Temperature for 15 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
12. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
13. Read the absorbance at 450 nm with a microplate reader.

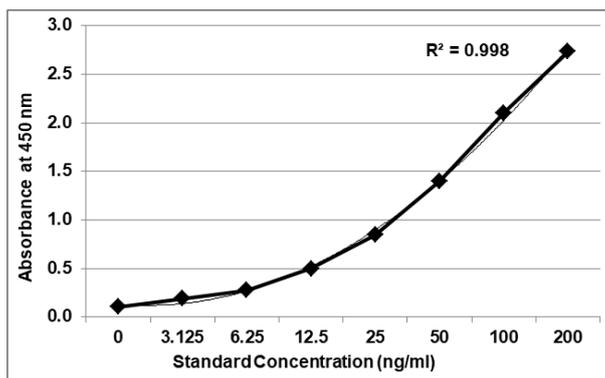
Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Using semi-log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown Human IgA concentrations, find the unknowns 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 cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.102	0.111	0.107	0.1	--
3.125	0.199	0.179	0.189	3.1	97.9
6.25	0.288	0.267	0.278	5.9	94.5
12.5	0.503	0.499	0.501	13.0	104.0
25	0.857	0.830	0.843	24.9	99.6
50	1.398	1.407	1.402	49.7	99.3
100	2.107	2.100	2.104	100.5	100.5
200	2.663	2.800	2.732	199.6	99.8

Typical Graph



Abs = absorbance at 450nm

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:

This kit has been validated as per guidelines in line with ICH Code for Harmonization of Biological Assays.

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 be 1.25 ng/ml

Specificity:

The assay detects both natural and recombinant human IgA. The cross-reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IgA positive sample. No crossreactivity or interference was detected.

Precision:

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (3.125 ng/ml), medium (25 ng/ml) and high (200 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<12%
Medium	<7%	<10%
High	<7%	<10%

Specificity/Cross-Reactivity:

In a sandwich ELISA cross reactivity can arise either as a false increase in Human IgA levels (positive cross reactivity) or as a false decrease in true Human IgA (negative cross reactivity). Positive cross reactivity was evaluated by assaying the sample as an unknown.

Recovery/ Interference Studies:

A number of buffer matrices have been evaluated by adding known amounts of the Human IgA preparation used to make the standards in this kit. As this assay is designed to minimize matrix interference most of these buffers yielded acceptable recovery defined as between 80-120%.

It is widely known that pH (less than 5.0 and greater than 8.5) as well as detergents like SDS and Tween can cause under-recovery. Very high concentrations of certain proteins can also interfere in accurate detection of Human IgA.

Users should qualify their sample matrices for accurate recovery by performing similar experiments. For example, this experiment can be performed by diluting one part of the 100 ng/ml standard provided with this kit into 3 parts of the sample matrix in question. Recovery should be between 80%-100% of the known concentration. Consult KRISHGEN at sales@krishgen.com if you have recovery difficulties in your matrix.

Hook Capacity:

The hook capacity, defined as that concentration that gives an absorbance reading less than the 200 ng/ml standard was ~200 ng/ml.

Safety Precautions:

- **This kit is For Research 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.
- 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.



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