KRIBIOLISA™ Human Serum Albumin (HSA) ELISA

REF : KBBP07

Ver 4.3

IVT

Enzyme Immunoassay for the measurement of Human Serum Albumin (HSA) in biological preparations

IVT	For In-Vitro Testing Use	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF **KBBP07** Σ **96 tests**

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Introduction:

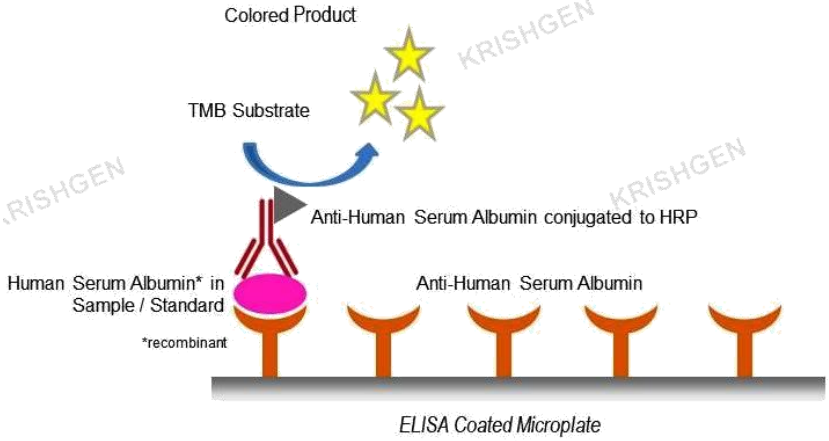
A variety of products, manufactured using the biotechnology route such as cell or tissue culture may result in residual contamination of the end-product. Trace contamination of HSA in pharmaceutical and other biological products is one such area. Such trace contaminants may lead to potential health hazards in humans, especially when the product is intended for therapeutic use.

Intended Use:

This generic KRIBIOLISA™ Human Serum Albumin (HSA) ELISA kit is intended in determining the presence of Human Serum Albumin contamination in various products that are manufactured through the biotechnology route. The kit has been validated successfully for testing of final product for HSA in variety of biological preparations regardless of growth and purification process.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to Human Serum Albumin are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human Serum Albumin present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-Human Serum Albumin antibody is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Serum Albumin in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

Part	Description	Qty
Anti-HSA Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Human Serum Albumin monoclonal antibody.	1 x 96 wells
HSA Standard	Recombinant Human Serum Albumin in a buffered protein base and preservative 0.02% methylisothiazolone and 0.02% bromonitrodioxane Lyophilized (1ug/ml)	2 vials
(1X) Standard Diluent	Buffered protein base with preservative 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	10 ml
Anti-Human Serum Albumin:HRP Conjugate	Anti-Human Serum Albumin conjugated to Horseradish Peroxidase with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with preservative 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	2 x 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. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (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. Store main kit components at 2-8°C.
2. Store HSA standards and detection antibody at -20°C. Upon thawing, aliquot standards and antibody into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. All the reagents provided may be harmful if ingested, inhaled or absorbed through the skin.
2. For In-Vitro Testing use only.

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. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent (1X) to obtain a concentration of 1ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Prepare **Standards** as per the below table. Use the Standard Diluent (1X)as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
1000 ng/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit + 1ml of Standard Diluent (1X)
100 ng/ml	Standard No.7	100 ul Reconstituted Standard (1ug/ml) + 900 ul Standard Diluent (1X)
50 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent (1X)
25 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent (1X)
12.5 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent (1X)
6.25 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent (1X)
3.125 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent (1X)
1.56 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent (1X)
0 ng/ml	Standard No.0	Only Standard 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 Serum Albumin. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Serum Albumin 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 Serum albumin 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 Human Serum Albumin.
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.
9. Samples where expected concentration is more than 100 ng/ml should be diluted. The diluent used should be compatible for accurate and optimal recovery. We recommend using our Sample Diluent. The preferred diluent is our Cat No#KBBP07dil is available in 100 ml, 500 ml, or 1 liter bottles. This is the same material used to prepare the kit standards. As the sample is diluted in the Sample Diluent its matrix recovery approaches that of the standards. This helps in reducing any inaccuracies caused by dilutional artifacts. Other prospective diluents should be qualified in the assay to demonstrate that they do not give elevated background and are not contaminated with human serum proteins. The diluent should also give acceptable recovery when spiked with known quantities of human serum albumin.
10. As the KRIBIOLISA™ Human Serum Albumin ELISA is an extremely sensitive assay for HSA, it is possible to inadvertently contaminate the kit reagents with various external sources of HSA. Such HSA impurities can arise from use of pipetting or other laboratory equipment or surfaces that have come into contact with more concentrated forms of HSA. For example, typically human serum contains ~40 mg/ml of HSA or about four million fold greater than the highest standard used in this kit. Airborne impurities from these same concentrated sources or from technician mucosal aerosols or dander will also easily contaminate the kit reagents and potentially give false values and/or poor assay reproducibility. It is recommended to take precautions to minimize impurities.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C

2. Pipette **100 ul** of prepared **Standards** or diluted **Samples** into the respective wells.

3. Cover the plate and incubate for 60 minutes at 37°C

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 **Anti-Human Serum Albumin:HRP Conjugate** into each well.

6. Cover the plate and incubate for 60 minutes at 37°C

7. 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.

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

9. Incubate the plate at 37°C for 30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

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

11. 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. Using Semi-Log 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 Serum Albumin 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 Human Serum Albumin Concentration. If samples were diluted, multiply by the appropriate dilution factor.

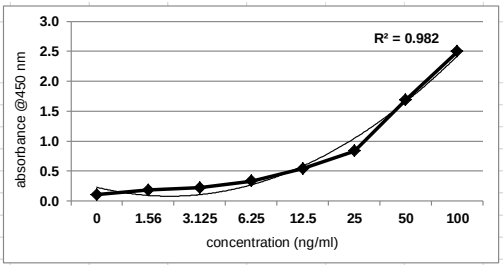
Software which is able to generate a cubic spline curve-fit or polynomial regression to 2nd order, is best recommended for automated results.

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.
- If the absorbance value is equivalent or higher than the 100 ng/ml standard.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs
0	0.112	0.108	0.110
1.56	0.185	0.189	0.187
3.125	0.218	0.228	0.223
6.25	0.327	0.338	0.333
12.5	0.534	0.546	0.540
25	0.823	0.853	0.838
50	1.768	1.612	1.690
100	2.452	2.545	2.499

Typical Graph



Abs = Absorbance

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 ng/ml

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 (12.5 ng/ml) and high (100 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 HSA levels (positive cross reactivity) or as a false decrease in true HSA (negative cross reactivity). Positive cross reactivity was evaluated by assaying the sample as an unknown.

Negative cross reactivity was evaluated by spiking 10 ng/ml of HSA into each of the potential cross reactants and dividing the recovered value by 10 ng/ml. None of the materials below showed either type of cross reactivity. The antibodies used in this kit have been affinity purified to minimize cross reactivity. However cross reactivity has not been extensively evaluated in this kit.

It is strongly recommended that each user test their particular sample matrix material for cross reactivity in a similar experiment.

Substance	% Cross-Reactivity
Bovine Albumin	Not detectable
Mouse Serum	Not detectable

Recovery/ Interference Studies:
A number of buffer matrices have been evaluated by adding known amounts of the HSA 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 HSA.

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:
Increasing concentrations of HSA greater than 100 ng/ml were assayed as unknowns. The hook capacity, defined as that concentration that gives an absorbance reading less that the 100 ng/ml standard was ~100 ug/ml.

Safety Precautions:

- **This kit is for In-vitro Testing Use only**. It is not for 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 at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide 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.

References:

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SYMBOLS KEY

MTP	Anti-HSA Coated Microtiter (12X8 wells)
STD	HSA Standard Lyophilized
HRP CONJ	Anti-Human Serum Albumin:HRP Conjugate
1X STD DIL	(1X) Standard Diluent
1X SAMP DIL	Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature