KRIBIOLISA™ Bovine Serum Albumin (BSA) ELISA

REF : KBBP10

Ver 4.3

RUO

Enzyme Immunoassay for the Quantitative Determination of Serum Albumin, BSA in Bovine 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**

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

REF KBBP10 **Σ 96 tests**

KRISHGEN BioSystems For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

Introduction:

The GENLISA ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a competitive ELISA technique.

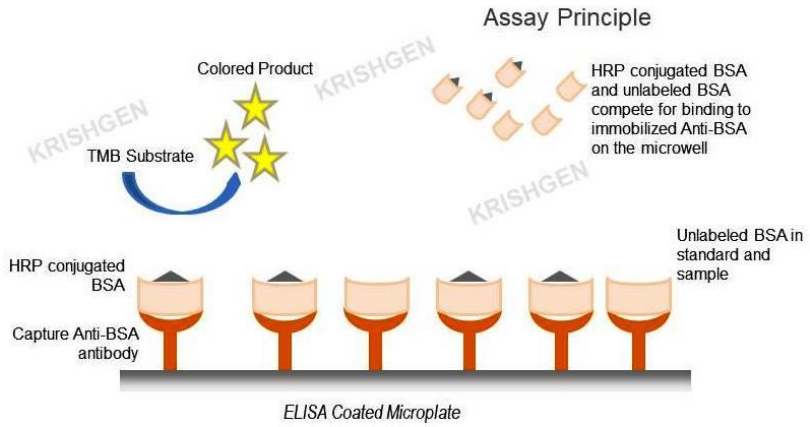
Intended Use:

The KRIBIOLISA™ Bovine Serum Albumin (BSA) ELISA kit is used as an analytical tool for quantitative determination of Bovine Serum Albumin, BSA serum, plasma and other biological samples.

Note: The KRIBIOLISA™ BSA ELISA is a very sensitive assay detecting only ~80 ng BSA per ml sample material. It is recommended to use disposable reagent containers for pipetting the conjugate. Make sure that the glassware and plastic material used for buffer preparation and reagent handling are absolutely free of BSA.

Principle:

The method employs competitive enzyme-linked immunosorbent assay (ELISA) technique to assay the level of Serum Albumin, BSA in samples. Standards or Samples compete with the HRP Conjugated BSA to form a complex with the Serum Albumin antibody. Wells are washed to remove the excess conjugate. After incubation and a washing step TMB Substrate, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the Bovine Serum Albumin (BSA) in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.



Materials Provided:

1. Anti-BSA Coated Microtiter Plate (8 x 12 wells) - 1 no
2. BSA Standard (lyophilized, concentrated, 10 ug/ml) - 2 vials
3. BSA:HRP Conjugate (concentrated) - 60 ul
4. Sample Diluent 1 - 20 ml
5. Sample Diluent 2 - 20 ml
6. HRP Conjugate Dilution Buffer - 10 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 pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator

8. Timer.

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned 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 Use Only.

Sample Preparation and Storage:

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

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
   Note: *Grossly hemolyzed samples are not suitable for use in this assay.*
4. **Adherent and Suspension Cell Culture:** Use three T25 flasks or one T75 flask for cell culture, the number of cells (1x107);
   1. Suspension cell: centrifuge at 2500 rpm at 2-8°C for 5 minutes; collect clarified cell culture supernatant;
   2. Adherent cell: collect supernatant directly; centrifuge at 2500 rpm at 2-8°C for 5 minutes; collect clarified cell culture supernatant for immediate detection or store it separately at -80°C.

   Cell Lysate Preparation: Two types of cell lysates are specified below.
   1. Suspension Cell Lysate : Centrifuge at 2500 rpm at 2-8°C for 5 minutes; then add pre-cooling PBS into collected cell and gently mix. Recollect cell by repeating centrifugation. Add 0.5 - 1 ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add suitable protease inhibitor (e.g. PMSF, working concentration: 1 mmol/l). Lyse the cell on ice for 30 min - 1 hr. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by ultrasonic cell disruptor (300W, 3~5 s/time, 30s intervals, 4-5 times) or ultrasonic generator (14 um for 30s). At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube and stored at -80°C.
   2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS once. Then, add 0.5 - 1 ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add the suitable protease inhibitor (e.g. PMSF, working concentration: 1 mmol/l). Scrape adherent cell gently with a cell scraper. Add the cell suspension into centrifugal tube. Lyse the cell on ice for 30 min - 1 hr. During lysate process, use the tip for pipetting or inte rmittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by ultrasonic generator (14 um for 30s) or ultrasonic cell disruptor (300W, 3~5 s/time, 30s intervals, 4-5 times). At the end of lysate/ultrasonic disruption, centrifuge at 10000rpm at 2-8℃ for 10 minutes. Then, the supernatant is added into EP tube and stored at -80°C.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided sample diluent, and several trials may be necessary. The test sample must be well mixed with

the sample diluent. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with the Sample Diluent.

Please refer to the following table of recommended dilution ratio for BSA samples for reference.

Dilution Fold	Sample	Sample Diluent 1	Sample Diluent 2	Total Diluted Sample Volume
1/2	60 ul	60 ul	---	120 ul
1/5	24 ul	96 ul	---	120 ul
1/10	12 ul	108 ul	---	120 ul
1/20	6 ul	114 ul	---	120 ul
1/50	3 ul	---	47 ul	50 ul + 100 ul Sample Diluent 1
1/100	3 ul	---	177 ul	180 ul + 120 ul Sample Diluent 1
1/1000	2 step dilution. Create a 50 fold dilution and then make a 20 fold dilution Sample diluent 2 is used throughout the dilution.			
1/10000	2 step dilution. Create a 100 fold dilution and then make a 100 fold dilution using Sample diluent 2 is used throughout the dilution.			
1/100000	3 step dilution. Create a 50 fold dilution and then make a 20 fold dilution. Finally create a 100 fold dilution using Sample diluent 2 is used throughout the dilution.			

Note: The volume in each dilution is not less than 3 ul. Dilution factor should be within 100 fold. Mix well during dilution and avoid foaming

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 **Wash Buffer (1X) 500 ml;** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**.
4. **BSA:HRP Conjugate Working Solution** - Dilute the HRP-labeled BSA with HRP Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 ul of HRP-labeled BSA into 99 ul of HRP Dilution Buffer).
5. **Standards Preparation**: Reconstitute original BSA Standard with 0.5 ml of Sample Diluent 1. Keep the standard for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
10000 ng/ml	Standard No.8	300 ul Reconstituted Original Standard + 300 ul Sample Diluent 1
5000 ng/ml	Standard No.7	300 ul Standard No.8 + 300 ul Sample Diluent 1
2500 ng/ml	Standard No.6	300 ul Standard No.7 + 300 ul Sample Diluent 1
1250 ng/ml	Standard No.5	300 ul Standard No.6 + 300 ul Sample Diluent 1
625 ng/ml	Standard No.4	300 ul Standard No.5 + 300 ul Sample Diluent 1
312 ng/ml	Standard No.3	300 ul Standard No.4 + 300 ul Sample Diluent 1
156 ng/ml	Standard No.2	300 ul Standard No.3 + 300 ul Sample Diluent 1
0 ng/ml	Standard No.1	300 ul Sample Diluent 1 only

6. **Prepared Standard-HRP-Antigen Complex:**
   a) When standards are not tested repeatedly: Take 55 ul of each prepared Standard and 55 ul BSA:HRP Conjugate Working Solution and mix them well in an Eppendroff tube for later use.
   b) When standard are tested in duplicate: Take 110 ul of each prepared Standard and 110 ul BSA:HRP Conjugate Working Solution and mix them well in an Eppendroff tube tube for later use.
   Take 110 ul of diluted sample and 110ul BSA:HRP conjugate working Solution and mix well in a eppendroff tube for later use.
7. **Prepared diluted Sample-HRP-Antigen Complex:**
   a) When samples are not tested repeatedly: Take 55 ul of each diluted Sample and 55 ul BSA:HRP Conjugate Working Solution and mix them well in an Eppendroff tube for later use.
   b) When samples are tested in duplicate: Take 110 ul of each diluted Standard and 110 ul BSA:HRP Conjugate Working Solution and mix them well in an Eppendroff tube tube for later use.

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 Bovine Serum Albumin, BSA. High Dose Hook Effect is due to excess of antibody for very high concentrations of Bovine Serum Albumin, BSA present in the sample.
3. Bovine Serum Albumin, BSA concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Bovine Serum Albumin, BSA.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. 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.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

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. To minimize noise / high blanks, it is recommended to wahs the plate 2 times as per Step (5) below. Tap and wipe dry.

2. Add **100 ul prepared Standard-HRP-Antigen Complex** to standard wells.

3. Add **100 ul diluted Sample HRP-Antigen Complex** to sample wells.

4. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

5. Aspirate and wash plate 4 times with diluted 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.

6. Pipette **100 ul TMB Substrate** in all the wells.

7. Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision.

8. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

9. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

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 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 Bovine Serum Albumin, BSA 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 Bovine Serum Albumin, BSA concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL (2^{nd} order) is best recommended for automated results.

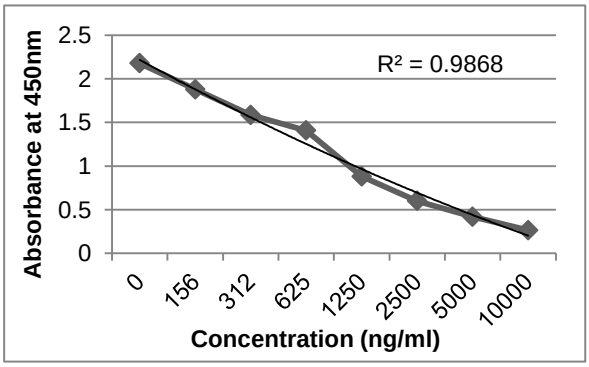
Note:

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.

Typical Data

Standard Concentration ng/ml	Absorbance 1	Absorbance 2	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	2.214	2.139	2.177	0	100.00
156	1.854	1.902	1.878	156.7	100.45
312	1.588	1.582	1.585	356.4	114.23
625	1.415	1.398	1.407	516.3	82.61
1250	0.872	0.888	0.880	1389.6	111.17
2500	0.587	0.612	0.600	2593.3	103.73
5000	0.423	0.412	0.418	4571.7	91.43
10000	0.260	0.273	0.265	10251.4	102.51

Typical Graph



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. Please view the details herein below.

Standard Calibration Range:
156 ng/ml - 10000 ng/ml

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 94 ng/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of BSA. No significant cross-reactivity or interference between BSA and analogues was observed.

Recovery
Matrices listed below were spiked with certain level of BSA and the recovery rates were calculated by comparing the measured value to the expected amount of BSA in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	85-103	96
EDTA Plasma(n=5)	85-102	95
Heparin Plasma(n=5)	85-101	93

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, medium and high 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	<11%	<11%
High	<11%	<11%

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of BSA and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	92-100%	88-104%	89-105%
EDTA Plasma(n=5)	86-99%	82-90%	87-100%
Heparin Plasma(n=5)	84-100%	87-101%	80-104%

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 Bovine 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	Standard No.1 Standard No.1			
1B 2B	Standard No.2 Standard No.2			
1C 2C	Standard No.3 Standard No.3			
1D 2D	Standard No.4 Standard No.4			
1E 2E	Standard No.5 Standard No.5			
1F 2F	Standard No.6 Standard No.6			
1G 2G	Standard No.7 Standard No.7			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2024

THANK YOU FOR USING A KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	Coated Microtiter Plate (8x12 wells)
STD	Standard
HRP CONJ	Conjugate Horseradish Peroxidase
HRP DIL	HRP Conjugate Dilution Buffer
SAMP DIL 1	Sample Diluent 1
SAMP DIL 2	Sample Diluent 2
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