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KRIBIOLISA™ Protein A ELISA

REF : KBBP12

Ver 3.1

RUO

Enzyme Immunoassay for the quantitative determination of Protein A in biological solutions.

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

REF KBBP12 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 Protein A ELISA is a quantitative, simple and will aid in monitoring the purification process as well as screening product prior to its use.

Intended Use:

This kit is used for determination of natural and recombinant constructs of Protein A ligand contamination, in various antibody products that are purified through Protein A chromatographic techniques.

Principle:

This assay is based on the principle of Sandwich ELISA. Samples containing Protein A are reacted with anti-Protein A:HRP antibody simultaneously in the microtiter wells already coated with affinity purified capture anti-Protein A antibody. This immunological reaction results in formation of a sandwich complex of solid phase antibody-Protein-enzyme labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure (see Assay procedure section mentioned below). TMB substrate is then added. Color develops proportionally to the amount of Protein A in the samples. Color development is stopped by addition of stop solution. Absorbance is measured at 450 nm

Materials Provided:

1. Anti-Protein A coated Microtiter plate (96 wells) - 1 no
2. Protein A Standards, (0.3ml/vial) - 0, 125, 500, 1000, 2000, & 4000 pg/ml
3. Anti-Protein A:HRP Conjugate - 12 ml
4. Sample Diluent - 30 ml
5. Dissociation Buffer - 12 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. 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. Standard 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 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:

For Cell Culture Supernatant **–** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

Preparation Before Use:

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

Samples should be diluted using the Sample Diluent provided. Then add one volume of the Disassociation Buffer to the diluted samples and incubate for 5 mins at Room temperature.

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); dilute 50 ml of 20X Wash Buffer in 950 ml of DI water.

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. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Protein A.
3. It is recommended that all Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to erroneous results in the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

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

2. Add **100 ul** of **Standards** or **Samples** into the respective wells.

3. Cover the plate and incubate for 30 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. Pipette without delay in the same order **100 ul** of **Anti-Protein A: HRP Conjugate** into each well.

6. Cover the plate and incubate for 30 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 10 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 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 Protein A 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 Protein A Concentration. If samples were diluted, multiply by the appropriate dilution factor.

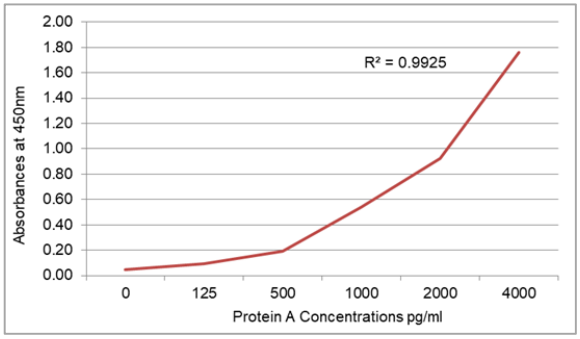
Software which is able to generate a polynomial regression (2nd order) or a cubic spline curve-fit is best recommended for automated results.

Note:
To estimate the amount of Protein A residue, the concentration of Protein A may also be reported in ppm to the concentration of the antibody. Example: the concentration of Protein A is detected at 500 pg/ml in an antibody with a concentration of 1 mg/ml. Then the antibody has residual Protein A of 0.5 ppm.

Typical Data

Std (pg/ml)	Abs#1	Abs#2	Mean
0	0.046	0.042	0.044
125	0.091	0.093	0.092
500	0.191	0.194	0.193
1000	0.538	0.547	0.543
2000	0.905	0.943	0.924
4000	1.718	1.803	1.761

Typical Graph



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.

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.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

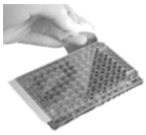
Components NOW (2-8ºC) → 30 mins RT Before Use → 

Thaw at Room Temperature (18-24ºC) → USE

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components RESULTS → AVOID → THAW (22±4ºC) → COOL (2-8ºC) → THAW (22±4ºC) → AVOID → NO CHANGE IN

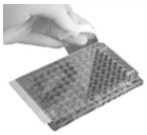
3. Pipette **100 ul Standards** / **Samples** into the respective wells.

4. Cover plate  and incubate for 

at 37°C.

5. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

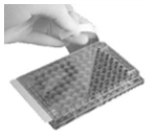
6. Pipette **100 ul Anti Protein A:HRP** into each well.

7. Cover plate  and incubate for 

at 37°C.

9. Aspirate and wash wells 4 times with **Wash Buffer (1X).**

10. Pipette **100 ul TMB Substrate** into each well.

11. Cover plate  and incubate for 

at 37°C.

12. Pipette **100 ul Stop Solution** into each well.

13. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Protein A equivalent
1A 2A	zero std zero std			
1B 2B	125 pg/ml 125 pg/ml			
1C 2C	500 pg/ml 500 pg/ml			
1D 2D	1000 pg/ml 1000 pg/ml			
1E 2E	2000 pg/ml 2000 pg/ml			
1F 2F	4000 pg/ml 4000 pg/ml			
1G 2G	*Sample*			
1H 2H	*Sample*			
3A 4A	*Sample*			
3B 4B	*Sample*			

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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	**Anti-Protein A Microtiter Plate (12X8 wells)**
STD	**Protein A Standard**
HRP CONJ	**Conjugate Horseradish Peroxidase**
SAMP DIL	**Sample Diluent**
WASH BUF 20X	**Wash Buffer (20X)**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalogue Number**
	**Expiration Date**
	**Storage Temperature**