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KRIBIOLISA™ Protein A ELISA

REF : KBBP11

Ver 2.2

RUO

Immunoassay for the measurement Natural & Structurally conserved Recombinant Protein A in samples containing Human immunoglobulin

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 **KBBP11** **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 preparations 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 antibody coated on the microtitre plates. The wells are washed to remove any unbound reactants as per the wash procedure. Anti-Protein A HRP antibody is added to the wells and incubated. . The wells are washed to remove any unbound reactants as per the wash procedure. simultaneously in the microtiter wells already coated with affinity purified capture anti-Protein A antibody. The TMB Substrate is then added and the wells incubated. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of Protein A present in the wells.

Materials Provided:

1. Anti-Protein A coated Microtiter plate (96 wells) - 1 no
2. Protein A Standards, (500 ul/vial) – 0, 62, 125, 500, 1000, 2000 and 4000 pg/ml
3. Anti-Protein A:HRP Conjugate - 12 ml
4. (20X) Wash Buffer - 25 ml
5. (1X) Sample Diluent - 50 ml
6. Protein A Dissociation Buffer - 12 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. Deionised (DI) water
4. Wash botte 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.
3. To make Wash Buffer (1X), dilute 25ml of Wash Buffer (20X) in 475 ml of DI water.

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 Protein A. High Dose Hook Effect is due to insufficient excess of antibody for very high concentrations of Protein A 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 Protein A 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 Protein A.
4. All Standards, Controls and Samples should be assayed atleast 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 all samples that need to be analyzed with Sample diluent. Add one volume of dissociation buffer to diluted samples and incubate at Room Temperature for 5 minutes.

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 100 ul of **Standards** and **Samples** into the respective wells and incubate at RT for 60 minutes.

3. 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.

4. Pipette out 100 ul of **Anti-Protein A:HRP Conjugate** into each well.

5. Cover the plate and incubate at RT for 30 minutes.

6. 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.

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

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

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

10. 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 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.

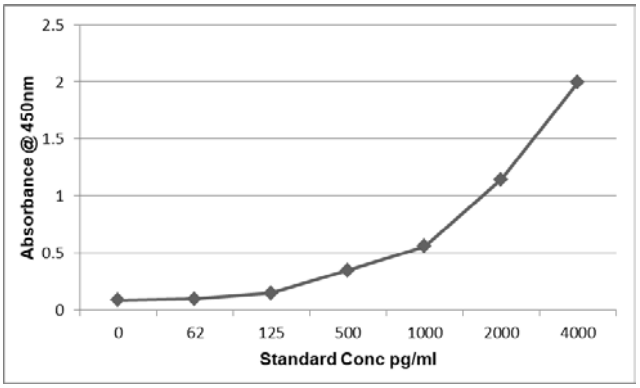
Software which is able to generate a cubic spline curve-fit or a polynomial curve (2^{nd} order) is best recommended for automated results.

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

Typical Data

Standard Concentration (pg/ml)	Mean Abs
0	0.087
62	0.098
125	0.150
500	0.346
1000	0.556
2000	1.145
4000	1.993

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 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 64 pg/ml.

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

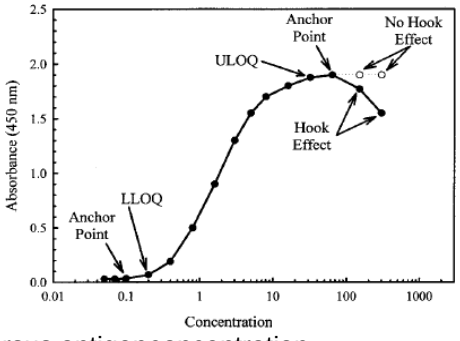
Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Protein A 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:
62 pg/ml – 4000 pg/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 antigenconcentration.

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

Increasing concentrations of Protein A >10,000 pg/ml were assayed as unknowns. **The hook capacity yielding an absorbance reading less than the 4000 pg/ml standard was ~8000 pg/ml.**

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.
- 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.
- 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	pg/ml Protein A concentration
1A 2A	Zero Std Zero Std			
1B 2B	62 pg/ml 62 pg/ml			
1C 2C	125 pg/ml 125 pg/ml			
1D 2D	500 pg/ml 500 pg/ml			
1E 2E	1000 pg/ml 1000 pg/ml			
1F 2F	2000 pg/ml 2000 pg/ml			
1G 2G	4000 pg/ml 4000 pg/ml			
1H 2H	Sample Sample			

LIMITED WARRANTY

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