KRIBIOLISA® Protein L Ligand Detection Kit

REF : KBBP50

Ver 2.4

RUO

Enzyme Immunoassay for the Quantitative determination of Protein L Ligand Detection from medium containing immunoglobulin (Ig) or Ig-fragments

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 KBBP50 Σ 96 tests

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Introduction:

Protein L was first isolated from the surface of bacterial species Peptostreptococcus magnus and and is used as ligand in chromatography media for capture of Ig and Ig fragments. It consists of 719 amino acid residues. Protein L binds to the variable region of the kappa light chain of the antibody, without interfering with the antigen- binding site. This allows Protein L to bind a wider range of Ig classes and subclasses than other antibody-binding proteins. In addition, Protein L also binds antibody fragments such as Fabs, single-chain variable fragments and domain antibodies which contain the kappa light chain.

Sample preparation is required in order to separate the Ig or Ig-fragments from Protein L before running the assay with the kit. For mAbs and Fabs, the samples are prepared by boiling and centrifugation before incubation in the strip wells.
Note that for small Ig-fragments like sdAbs, this sample preparation procedure does not work. Additional optimization of the sample preparation is required for sdAbs.

Intended Use:

The KRIBIOLISA® Protein L Ligand Detection Kit is a quantitative enzyme-linked immunosorbent assay. This ELISA-kit has been designed to detect and quantify ligand leakage in eluates from Protein L affinity chromatography medium containing immunoglobulin (Ig) or Ig-fragments.

Materials Provided:

Part	Description	Qty
Anti-Protein L Ligand Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Protein L Ligand	1 x 96 wells
Protein L Ligand Standard	Standards prepared in a buffered protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane (lyophilized, 1 ug/ml)	2 vials
Anti-Protein L Ligand:HRP Conjugate concentrated	Anti-Protein L Ligand conjugated to Horseradish Peroxidase. concentrated (1 mg/ml)	2 vials
Detection Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane.	12 ml
Standard Diluent	Buffered protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane	10 ml
(1X) Assay Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane.	25 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersal < 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. Standard (mm/mm) graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. It is advisable to aliquot and store the Anti-Protein L Ligand:HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess Working

Anti-Protein L Ligand:HRP Conjugate after running your assay All reagents should be stored at 2 to 8^{0}C for stability.

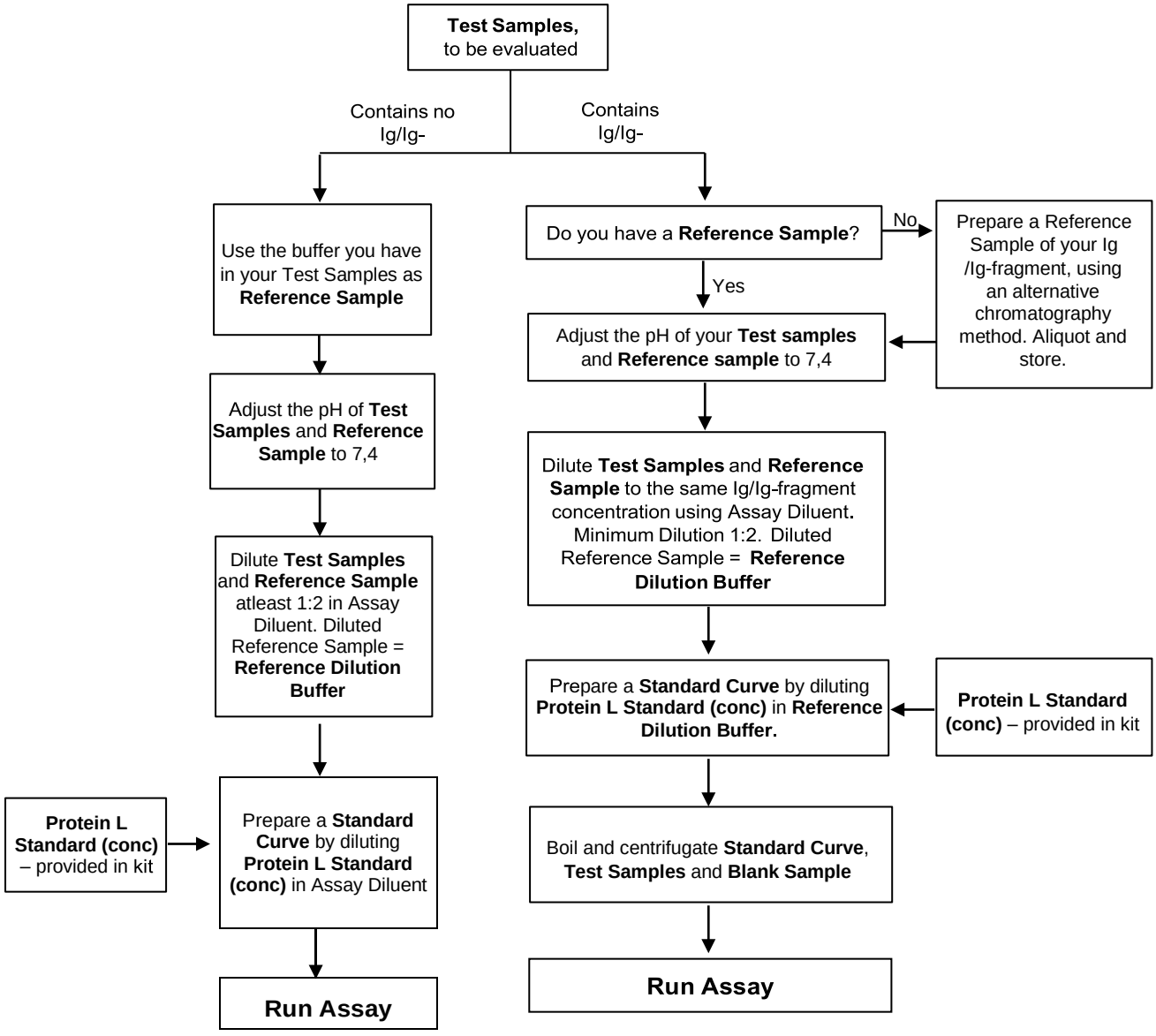
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive 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:

Schematic Overview



Reference Sample (Ig/Ig-fragment sample without Protein L)

As different target molecules, at different concentrations, may have different effect on the assay performance, there is a need to dilute the Protein L Standard (conc), provided in the kit in a Reference Sample. The reference sample should contain the same Ig or Ig-fragment in an equivalent buffer and at the same concentration as the Test samples to be assayed (i.e. elution samples from the Protein L affinity chromatography medium). Importantly, the Ig or Ig- fragments of the reference sample should be prepared by use of an alternative purification strategy

(i.e. not involving Protein L affinity chromatography medium, for example a suitable ion exchange column). Aliquot the Reference sample and store at suitable conditions, e.g. -20ºC or -80ºC, to be used for several different Protein L ELISA assays. If your Test samples do not contain any Ig or Ig-fragments, use your test sample buffer as Reference sample.

- If needed, adjust the pH of your reference sample to 7.4.
- Dilute the reference sample in Assay Diluent at least 1:2, to match the concentration in your samples.
- Use this solution (Reference dilution buffer) for dilution of the Protein L reference supplied in the kit, in order to get a standard curve (see below).

Sample pH and Dilution:

- Adjust the pH of all samples to approximately 7.4.
- Dilute all samples at least 1:2 in Assay Diluent. Make sure all samples contain equal amounts of target molecule (Ig/Ig-fragment), the same concentration as in your Reference dilution buffer (see above).
- Samples containing Ig or Ig-fragments need to be further prepared by boiling and centrifugation (see below). Samples without Ig or Ig-fragments may be used directly in the assay.

Sample Preparation:

This step is necessary only if your samples contain Ig or Ig-fragments. Samples without Ig or Ig-fragments may be used directly in the assay.

- Boil test samples, blank sample and reference standard curve samples. Use tubes with screw cap. Do not use vials with skirt if using a heating block.
- Ig-containing samples should be boiled for 15min. Samples containing Ig-fragment should be boiled for 1 hour.
- Centrifuge samples for 2 minutes.
- Carefully mix the samples, without disrupting the pellet, before adding them to the plate.

Note: The protocol for sample preparation may be optimized for different Ig-fragments by altering the boiling time and dilution. Generally, smaller target molecules need longer boiling time.

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 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 to obtain a concentration of 1 ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 50 ul of original **Standard (1 ug/ml)** with 450 ul of Standard Diluent to generate a **100 ng/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard No. 0).

Standard Concentration	Standard Vial	Dilution Particulars
1 ug/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 1 ml of Standard Diluent
100 ng/ml	Standard No.6	50 ul Reconstituted Standard (1 ug/ml) + 450 ul Standard Diluent
50 ng/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent
25 ng/ml	Standard No.4	250 ul Standard No.5 + 250 ul Standard Diluent
12.5 ng/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard Diluent
6.25 ng/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard Diluent
3.125 ng/ml	Standard No.1	250 ul Standard No.2 + 250 ul Standard Diluent
0 ng/ml	Standard No.0	Only Standard Diluent

5. **Working Anti-Protein L Ligand:HRP Conjugate** – **Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**

Use the Standards immediately upon reconstitution. Discard balance standard after use. Do not store them for further experiments.

Note: Make sure to always treat your Standard samples the same way as your Test samples. If your Test samples contain Ig or Ig-fragments and need boiling as sample preparation, your reference standard curve samples should be boiled too, at the same conditions.

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 Protein L. High Dose Hook Effect is due to excess of antibody for very high concentrations of Protein L present in the sample.
3. 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 L 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 Protein L.
5. It is recommended that all Standards and Samples be assayed in duplicates.
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. Pipette **50 ul** of **Standards** or **Samples** into the respective wells.

2. Cover the plate and incubate for 60 minutes at Room Temprature.

3. 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.

4. Pipette **100 ul** of **Working Anti-Protein L Ligand:HRP Conjugate** into the respective wells.

5. Cover the plate and incubate for 60 minutes at Room Temperature.

6. Aspirate and wash plate 4 times with **Wash Buffer (1X)** same as in step 3.

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

8. Incubate the plate at Room Temperature 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.

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

10. 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 Semi-Log 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 L ligand concentrations, find the unknown's Mean Absorbance value

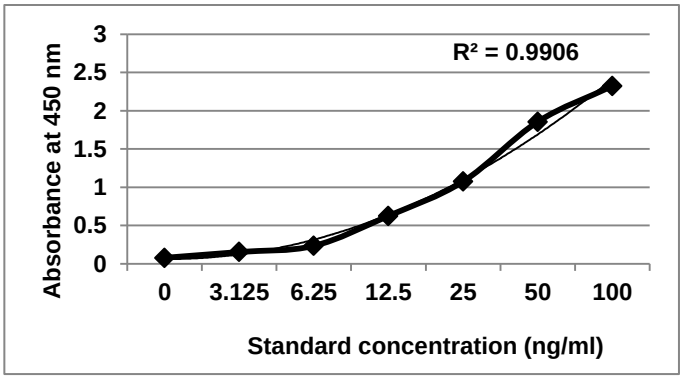
on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Protein L ligand Concentration. If samples were diluted, multiply by the appropriate dilution factor.
Software which is able to generate a cubic spline curve-fit or a polynomial curve (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 (representative only)

Standard Concentration (ng/ml)	Mean Abs	Interpolated Concentration	% Recovery
0	0.077	--	--
3.125	0.153	3.3	106.6
6.25	0.233	5.4	86.9
12.5	0.625	13.7	109.8
25	1.074	23.6	94.5
50	1.855	51.9	103.7
100	2.322	98.0	98.0

Typical Graph (representative only)



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

The measuring range of the kit is at least 3.125-100 ng/ml of Protein L for samples not containing Ig or Ig-fragments. The sensitivity of the assay may be reduced for samples containing Ig or Ig-fragments.

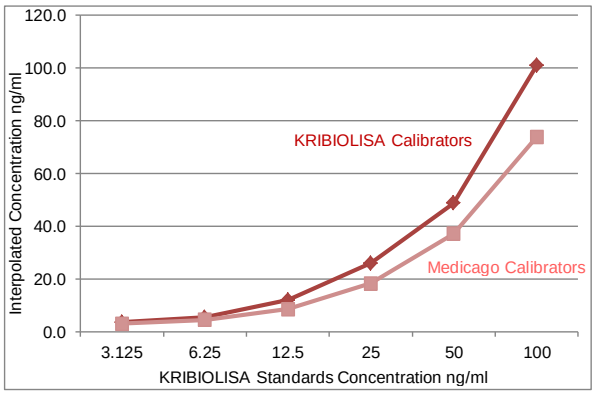
Comparator Performance

The KRIBIOLISA® Protein L Ligand ELISA was used to assess the performance of the kit in comparison to the Protein L Ligand Leakage ELISA from Medicago AB, Sweden.

Calibrator from the Medicago kit bearing catalog no #10-0027-1 was used as calibrator in the KRIBIOLISA® Protein L Ligand ELISA. The calibrator from the Medicago kit was diluted using the KRIBIOLISA® kit Assay Diluent and the recovery was assessed.

Medicago AB calibrator serially diluted Concentration (ng/ml)	Mean Abs	Interpolated Concentration (ng/ml)	% Recovery
3.125	0.120	3.2	101.1
6.25	0.164	4.6	73.1
12.5	0.321	8.7	69.8
25	0.736	18.3	73.3
50	1.442	37.3	74.5
100	2.207	74.0	74.0

KRIBIOLISA Interpolated Concentration (ng/ml)	Medicago Interpolated Concentration (ng/ml)
3.6	3.2
5.6	4.6
12.0	8.7
26.0	18.3
48.8	37.3
101.0	74.0



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SYMBOLS KEY

MTP	**Anti-Protein L Ligand Coated Microtiter Plate (12x8 wells)**
STD	**Protein L Ligand Standard, Lyophilized**
HRP CONJ	**Anti-Protein L Ligand:HRP Conjugate concentrated**
DETN DIL	**Detection Diluent**
20X WASH BUF	**(20X) Wash Buffer**
1X ASY DIL	**(1X) Assay Diluent**
STD DIL	**Standard Diluent**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**