ab325035 – Protein L Ligand Leakage Detection ELISA Kit

For the quantitative determination of Protein L Ligand Detection from medium containing immunoglobulin (Ig) or Ig-fragments.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325035

Storage and Stability: Store the whole kit, apart from Anti-Protein L Ligand:HRP Conjugate at 2-8°C immediately upon receipt. Please refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Storage
Anti-Protein L Ligand Coated Microtiter Plate	1 unit	2-8°C
Protein L Ligand Standard	2 vials	2-8°C
Anti-Proten L Ligand:HRP Conjugate	12 mL	-20°C
Detection Diluent	12 mL	2-8°C
(1X) Standard Diluent	10 mL	2-8°C
(1X) Assay Diluent	25 mL	2-8°C
(20X) Wash Buffer	25 mL	2-8°C
TMB Substrate	12 mL	2-8°C
Stop Solution	12 mL	2-8°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

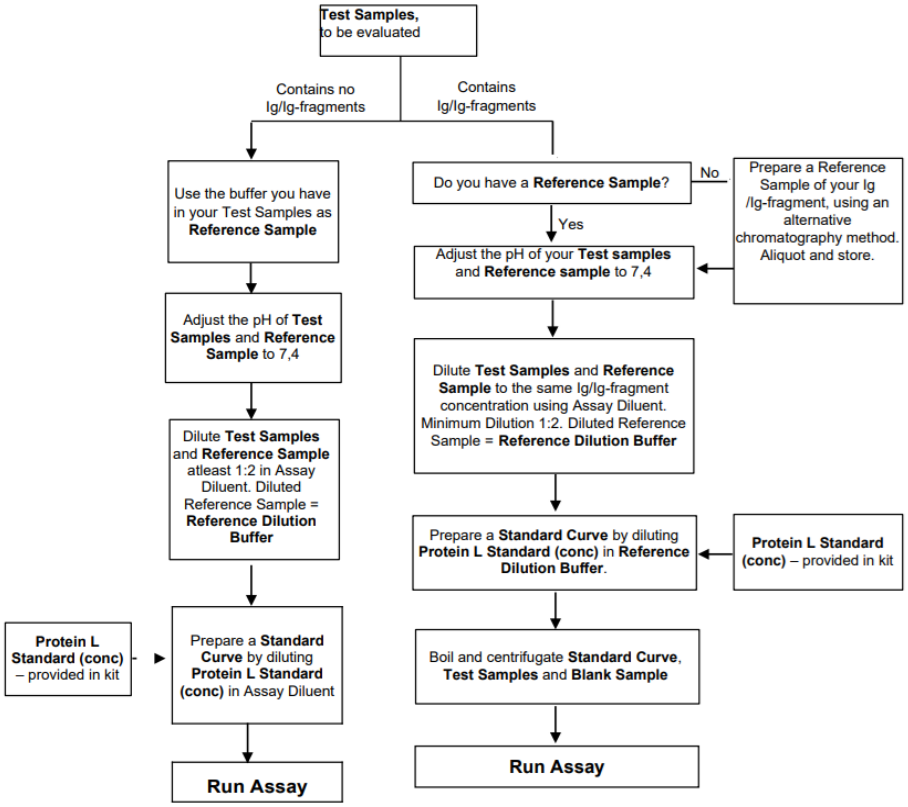
- Microtiter Plate Reader able to measure absorbance at 450 nm
- Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 µL to 1000 µL
- Deionized (DI) water
- Wash bottle or automated microplate washer
- Standard (mm/mm) graph paper or software for data analysis
- Timer
- Absorbent Paper

Handling and Storage Information

- 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°C for stability.
- All the reagents and wash solutions should be used within 12 months from manufacturing date.
- Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
- The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

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. -20°C or -80°C, 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 15 minutes. 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)

- Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
- Bring all reagents to Room temperature before use.
- To make Wash Buffer (1X); dilute 25 mL of 20X Wash Buffer in 475 mL of DI water.

Standards Preparation

1. Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent to obtain a concentration of 1 µg/ml. Keep the vial for 15 minutes with gentle agitation before making further dilutions.
2. Dilute 50 µL of original Standard (1 µg/ml) with 450 µL of Standard Diluent to generate a 100 ng/mL Standard Solution.
3. Prepare further Standards by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard #0).

∆***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.*

Standard Concentration	Standard Vial	Dilution Particulars
1 µg/mL	Reconstituted Standard	Lyophilized Standard provided in the Kit + 1 mL Standard Diluent
100 ng/mL	Standard#6	50 µL Reconstituted Standard (1 µg/ml) + 450 µL Standard Diluent
50 ng/mL	Standard#5	250 µL Standard#6 + 250 µL Standard Diluent
25 ng/mL	Standard#4	250 µL Standard#5 + 250 µL Standard Diluent
12.5 ng/mL	Standard#3	250 µL Standard#4 + 250 µL Standard Diluent
6.25 ng/mL	Standard#2	250 µL Standard#3 + 250 µL Standard Diluent
3.125 ng/mL	Standard#1	250 µL Standard#2 + 250 µL Standard Diluent
0 ng/mL	Standard#0	Only Standard Diluent

Procedural Notes

- In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
- 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.
- 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.
- 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.
- It is recommended that all Standards and Samples be assayed in duplicates.
- Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
- If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to the sensitivity of the assay being compromised.
- The plates should be read within 30 minutes after adding the Stop Solution.
- Make a work list in order to identify the location of Standards and Samples.

Assay Procedure

1. Pipette 50 µL of Standards or Samples into the respective wells.
2. Cover the plate and incubate for 60 minutes at Room Temperature.
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 µL of 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 4.

7. Add 100 µL 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 µL of Stop Solution. Wells should turn from blue to yellow in color.
10. 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 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 if the sample absorbance value is below the first standard or if the absorbance value is equivalent or higher than the 100 ng/ml standard.*

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For technical support contact information, visit: www.abcam.com/contactus

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