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Protein L ELISA Kit

Instruction for use

Contents

1. Product Introduction

Protein L can bind variable light chain (VL) of Kappa variants without interfering the antigen binding sites, which enables its applications in the capture of antibody and antibody fragments in chromatography process. Impacted by solutions and pH variation, Protein L ligands leakage will inevitably occur in chromatography process, leading to Protein L residue in antibody-based drugs. Bestchrom Protein L ELISA Kit is specially designed for the detection of Protein L ligands leakage in a highly sensitive manner, providing precise recognition towards Bestchrom Protein L Diamond resins ligands and therefore, enabling accurate/sensitive measurement of Protein L ligand leakage on various biologics using Protein L Diamond in purification.

2. Detection Principle

Protein L ELISA Kit can detect Protein L residue from samples via Two-site Sandwich ELISA approach. First dilute Protein L-containing sample with Sample Diluent. Isolate Protein L and sample antibody by boiling. After centrifugation, add sample to and let it react with the polycolonal anti-Protein L pre-coated ELISA Microplate. A secondary anti-Protein L antibody labeled directly with horse radish peroxidase (HRP) enzyme is simultaneously reacted forming as sandwich complex of solid phase antibody-Protein L-enzyme-linked antibody. After two wash steps to remove unbound reactants, the strips will then react with tetramethyl benzidine (TMB) substrate, producing a color change (from colorless to blue). It will finally turn to yellow after adding Stop Solution. The more Protein L ligands residue exist in sample, the darker the color will be.The Detect optical density (OD) at 450_{nm} and 650_{nm}. OD shall be positively related to Protein L contents in sample.

3. Assay Protocol

- Kit preparation: Bring all reagents to room temperature. See table 1 for kit components.

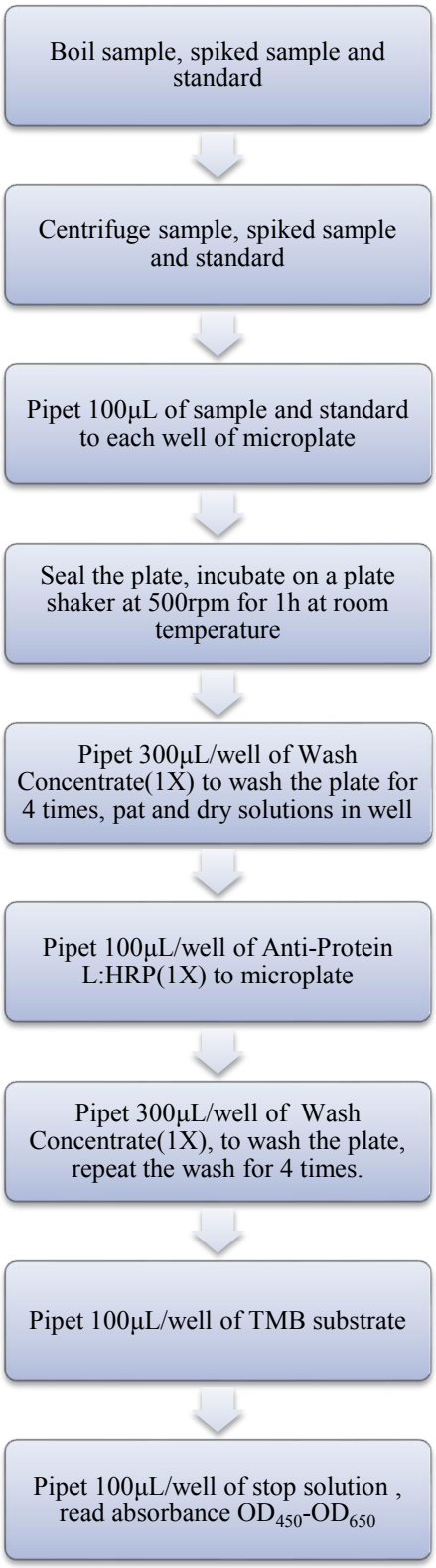
Table 1 kit components

S/N	Component	Pack size	Description
1	Anti-Protein L pre-coated Microplate	8 wells x12 strips	Dismantlable, depend on practical requirement
2	6# Standard	8 vial×1mL	0 ng/mL,0.16 ng/mL, 0.31 ng/mL,0.63 ng/mL, 1.25 ng/mL,2.5 ng/mL, 5 ng/mL,10ng/mL
3	Anti-Protein L:HRP(100X)	150μL/vial	Enzyme-linked antibody, use Anti-Protein L:HRP Diluent to get 1X dilution
4	Sample Diluent	2 bottle×25mL/ bottle	Sample diluent
5	Stop Solution	12mL/bottle	Acid solution, corrosive ——Stop solution

S/N	Component	Pack size	Description
6	TMB	12mL/bottle	Solution containing 3,3',5,5' – tetramethylbenzidine and H_2O_2
7	Wash Concentrate(10X)	50mL/bottle	Use deionized water to get 1X dilution

- Reagent preparation:
  - Anti-Protein L: HRP(100X): Use Sample Diluent to get 1X dilution.
  - Wash Concentrate(10X): Use ultra-purified water to get 1X dilution.
- Sample preparation:
  - Standard: Before using, mix with a vortex mixer, centrifuge sample.
  - Sample to be tested: Adjust pH of all samples to about 7.4. Dilute with Sample Diluent and set aside for use. It is recommended to dilute sample to 1mg/mL and below. Sample volume shall be no less than 400μL after dilution.
  - Spiked sample: Spiked sample recovery rate detection is an important criterion for the applicability and methodological validation of the experimental system. Volume of spiked sample shall be no less than 400μL.
  - Boil sample. Recommended boiling period for Ig-containing samples is 20min. Recommended boiling period for Ig fragments-containing samples is 1 hour.
  - Perform centrifuging on boiled sample, spiked sample and standard at 12000g for 5 min.
  - Note: Sample preparation methods can be optimized by adjusting boiling period and dilution ratio based on different Ig fragments. Smaller target molecules usually require longer boiling period.
- Experimental procedure:
  - Bring kit components to room temperature and carry out all operations at room temperature. It is recommended to run sample in replicate.
  - Add sample:
    1) Pipet 100μL of boiled sample/spiked sample/standard to well of the Anti-Protein L pre-coated Microplate (100μL/ well). Allow duplicates of each sample and Standard to be measured in the assay (parallel). Incubate on a plate shake (500rpm) for 1 hour.
    2) Wash: Shake the microplate to remove any remaining liquid on it. Pipette 300μL of Wash Concentrate (1X) to each well, repeat the process for 4 times.
    3) Pipet 100μL of Anti-Protein L: HRP(1X) to well of the Anti-Protein L pre-coated Microplate (100μL/ well). Incubate on a plate shake (500rpm) for 1 hour.
    4) Wash: Shake the microplate to remove any remaining liquid on it. Pipette 300μL of Wash Concentrate (1X) to each well, repeat the process for 4 times.
    5) Coloring: Pipet 100μL of TMB substrate to each well, incubate at room temperature in dark for 10 min until the color of max concentration standard to dark blue.
    6) Ending: Add 100μL of Stop Solution to each well (Color turns from blue to yellow).
    7) Read absorbance (OD) at 450_{nm} and 650_{nm}. Calculate the difference of OD between 450_{nm} and 650_{nm}.

- Workflow of leached Protein L detection

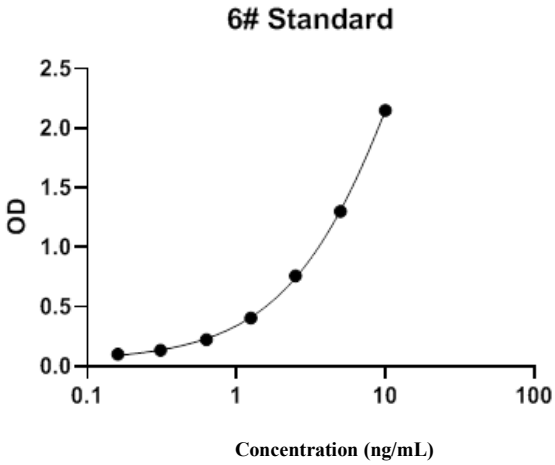


4. Data Analysis

- The calculation of OD: For each standard or sample, $OD=OD_{450nm}-OD_{650nm}$, OD should be the average value (mean) of replicates of standards/samples. Use microplate reader software or other softwares such as GraphPad Prism to analysis data. Using standard concentration as abscissa(X) and standard absorbance concentration as ordinate(Y), get standard curve. It is recommended to use four-parameter curve fitting equation. Calculate Protein L content in sample and spiked sample using the fitted standard curve. Pay attention to the dilution ratio.

➢ For standard curve of 6# Standard

6# Standard (ng/mL)	Average OD
10	2.149
5	1.301
2.5	0.759
1.25	0.4055
0.63	0.224
0.31	0.134
0.16	0.102
0	0.042



5. Product Performance

- Spiked experiment and recovery validation: Evaluation of Protein L with the existence of Kappa IgG. Since Kappa IgG has good affinity to Protein L, mix 1mg/mL of Kappa IgG with 6# Standard (in different concentrations). Get measured concentration of sample according to standard curve of the kit. Calculate the expected percentage value by dividing the measured concentration by the expected concentration.
- Precision: Intra and inter-assay precision.

➢ Intra-assay precision:

Conduct 16 repetitive detections to 3 Protein L -containing control samples via single experiment

#of Tests	6# Standard Concentration(ng/mL)	%CV
16	8	6.4
16	3	5.8
16	0.5	6.2

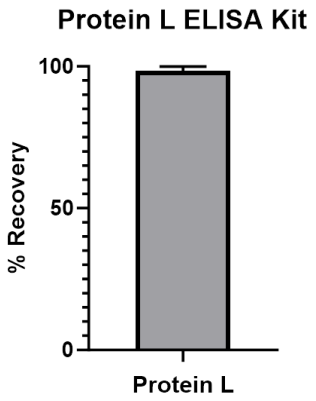
➢ Inter-assay precision:

Using 3 batches of Kits to detect the Protein L-containing control sample

#of Tests	6# Standard Concentration(ng/mL)	%CV
3	8	7.2
3	3	5.1
3	0.5	5.7

- Specificity: Protein L ELISA Kit can specifically recognize Protein L. The following picture shows evaluation of Protein L constructs.

  Get measured concentration of sample according to standard curve of the kit. Calculate the expected percentage value (n=9) by dividing the measured concentration by the expected concentration.



6. Precautions

- Before using, all components of kit shall be recovered to room temperature(20-24℃).
- It is recommended to dilute sample to a concentration level of 1mg/mL or below. It is recommended to boil Ig-containing samples for 20min and Ig fragments-containing samples for 1 hour. In case of abnormal yield, prolong boiling period to maximum 1 hour.
- HOOK effect: Within certain concentration range, concentration of analyte shows positive correlation to detection signal. However, when analyte concentration reaches to a certain level, signal will decrease despite of the increase of analyte concentration. This phenomenon gets its name “hook effect” for the hook shape of concentration/signal curve. When protein L concentration is excessively high, detection level can be lower than 10ng/mL. In this case, dilution of sample(Protein L) can usually solve the issue. Therefore, when detecting Protein L leakage, it is recommended to conduct dilution linear evaluation to eliminate the impact of “Hook effect”.
- For recovery detection by adding Protein L (with known content), accepted recovery range is usually from 80%-120%. Extreme pH or salinity might cause abnormal recovery. Under some conditions, high concentrated antibody will cause negative interference. In that case, please contact Bestchrom Technical Support Team for help.
- Avoiding using pipette tip to touch the bottom of microplate, to prevent any damage to pre-coating.
- After washing the ELISA microplate, tap it to dry it. Make sure no strip is falling out.
- During the reaction, to minimize the solution evaporation from microplate, it is recommended to

cover the ELISA microplate and Sample Treatment Plate using the covering membrane provided in ELISA Kit.

- Use the kit within its validity. When detecting Protein L leakage, it is necessary to use the right standard. Please avoid using reagents from different batches.
- Detection results variation might be caused by various factors, including experimental staff operation, the use of pipette, plate washing method, reaction time, contaminant in bottom of ELISA microplate, or temperature.
- This kit is for in vitro research experiments only, not for clinical diagnosis.
- Other needed reagents(not provided by kit): Deionized water, 1.5mL low-adsorption centrifuge tubes, high precision pipette & low adsorption pipette tip, tissue paper, microplate reader, microplate shaker(200-500rpm), software can be used for the fitting of four-parameter curve(e.g. GraphPad Prism).

7. Storage

Preserved at 2-8℃.

8. Ordering Information

Product	Cat.No.
Protein L ELISA Kit	EK004
Anti-Protein L pre-coated Microplate	EK004-01
6# Standard	EK004-02
Anti-Protein L:HRP(100X)	EK004-03
Sample Diluent	EK004-04
Stop Solution	EK004-05
TMB	EK004-06
Wash Concentrate(10X)	EK004-07