



# Hamster PLBL2 ELISA Assay Kit

Catalog Number: HPB93-K01

*For Research Use Only. Not for use in diagnostic procedures.*

*V. 4.1 (08 MAR 24)*

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## INTENDED USE

The PLBL2 test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring PLBL2 in hamster (CHO) cell samples. If the ELISA is to be used outside the intended use, the user may need to optimize for said use.

*For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at [www.EagleBio.com](http://www.EagleBio.com) or at 866-411-8023.*

## ASSAY PRINCIPLE

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the PLBL2 present in samples reacts with the anti-PLBL2 antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, the Detection Antibody, biotin conjugated anti-PLBL2, is added and complexes are formed. Following a wash step, the horseradish peroxidase (HRP) conjugated Streptavidin is added and complexes are formed. After another washing step, the complexes are assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of PLBL2 in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of PLBL2 in the test sample. The quantity of PLBL2 in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

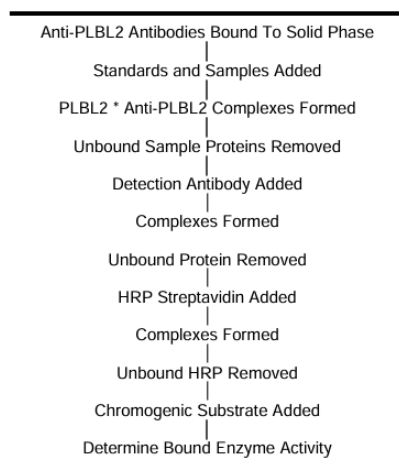


Figure 1

## LIMITATION OF PROCEDURE

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Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.



## MATERIALS PROVIDED

Component	Description	Preparation	Storage	Stability
<b>ELISA Microplate</b>	One plate of 12 removable 8 well strips, antibody coated	Ready to use	2-8°C, in a sealed foil bag with desiccant	Wither proper storage the plate strips are stable until the expiration date
<b>Detection Antibody 100X</b>	One vial of 150uL of 100X affinity purified antibody conjugated with biotin in a stabilizing buffer	Dilute 1/100 immediately prior to use.	2-8°C	The working solution should be diluted immediately prior to use and is stable up to one hour if stored in the dark. The 100X conjugate is stable until the expiration date.
<b>HRP-Streptavidin 100X</b>	One vial of 150uL of 100X Horseradish Peroxidase Conjugated antibody in a stabilizing buffer	Dilute 1/100 immediately prior to use	2-8°C in the dark	The working solution should be diluted immediately prior to use and is stable up to one hour if stored in the dark. The 100X conjugate is stable until the expiration date.
<b>Calibrator</b>	One vial of calibrator	Refer to Certificate of Analysis	2-8°C for lyophilized calibrator. Aliquoted and frozen if re-constituted. Avoid multiple freeze-thaw cycles.	The working standard Solutions should be prepared immediately prior to use
<b>Diluent Solution</b>	One 60mL bottle of 1X diluent buffer	Ready to use as supplied	2-8°C for both 1X working solution and 5x concentrate	The 1X working solution is stable until the expiration date.
<b>Wash Solution Concentrate</b>	One 50mL bottle of 20X wash solution	Dilute 1/20 to make 1X working solution	2-8°C for both 1X working solution and 20X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date.
<b>Chromogen-Substrate Solution</b>	One bottle of 12mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at PH 3.3.	Ready to use	2-8°C in the dark	Protect from light. The Substrate is stable until the expiration date.
<b>STOP Solution</b> <b><u>WARNING: Avoid contact with skin</u></b>	One 12mL bottle of 0.3 M sulfuric acid	Ready to use	2-8°C	The Stop Solution is stable until the expiration date



## MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2  $\mu$ L to 100  $\mu$ L) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtitre washer/aspirator
- Distilled or Deionized H<sub>2</sub>O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer
- Microplate Shaker

## SPECIMEN COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing. If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

## DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- CHO culture extract samples – Recommended starting dilution is 1/20. To prepare a 1/20 dilution of a sample, transfer 15  $\mu$ L of sample to 285  $\mu$ L of 1X diluent. This gives you a 1/20 dilution. Mix thoroughly.

## REAGENT PREPARATION

- **Bring all reagents to room temperature (16°C to 25°C) before use.**
- Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH<sub>2</sub>O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.
- Detection Antibody - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10  $\mu$ L Detection Antibody to 990  $\mu$ L of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.
- HRP-Streptavidin - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10  $\mu$ L HRP-Streptavidin to 990  $\mu$ L of



1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

- Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
- Hamster (CHO) PLBL2 Calibrator – Prepare according to the lot specific Certificate of Analysis.

## ASSAY PROCEDURE

1. All samples and standards should be assayed in duplicates.
2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

Pipette 100  $\mu$ L of

Standard 0 (0.0 ng/mL) in duplicate  
Standard 1 (0.31 ng/mL) in duplicate  
Standard 2 (0.63 ng/mL) in duplicate  
Standard 3 (1.25 ng/mL) in duplicate  
Standard 4 (2.50 ng/mL) in duplicate  
Standard 5 (5 ng/mL) in duplicate  
Standard 6 (10 ng/mL) in duplicate  
Standard 7 (20 ng/mL) in duplicate

3. Pipette 100  $\mu$ L of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate while shaking on a microplate shaker at 400rpm at room temperature for one hundred and twenty ( $120 \pm 2$ ) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100  $\mu$ L of appropriately diluted Detection Antibody to each well. Incubate while shaking on a microplate shaker at 400rpm at room temperature for twenty ( $20 \pm 2$ ) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100  $\mu$ L of appropriately diluted HRP-streptavidin to each well. Incubate while shaking on a microplate shaker at 400rpm at room temperature for twenty ( $20 \pm 2$ ) minutes. Keep plate covered in the dark and level during incubation.
10. Wash and blot the wells as described in Steps 5/6.
11. Pipette 100  $\mu$ L of TMB Substrate Solution into each well.



12. Incubate while shaking on a microplate shaker at 400rpm in the dark at room temperature for precisely ten (10) minutes. Keep plate covered in the dark and level during incubation.
13. After ten minutes, add 100  $\mu$ L of Stop Solution to each well.
14. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications

## **CALULATION OF RESULTS**

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the IgE concentration in original samples.

## **WARRANTY INFORMATION**

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.*