

ab291063 - Hamster (CHO) Phospholipase B-Like 2 (PLBL2) ELISA Kit

For Determination of PLBL2 in Hamster (CHO) Samples.
For research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab291063>

Storage and Stability

On receipt, entire assay kit should be stored at 4°C, protected from light. The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per this kit protocol insert.

Materials Supplied

Item	Quantity	Storage Condition
ELISA Micro Plate, antibody coated	12X8 unit	4-8°C
100X Detection Antibody	150 µL	4-8°C, store in dark
100X HRP-Streptavidin	150 µL	4-8°C, store in dark
Calibrator	1 vial	4-8°C
Diluent Solution	60 mL	4-8°C
Wash Solution Concentrate	50 mL	4-8°C
Chromogen Substrate Solution	12 mL	4-8°C, store in dark
STOP Solution	12 mL	4-8°C

Materials Required, Not Supplied

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

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squir bottle or Microtitre washer/aspirator
- Distilled or Deionized H₂O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer
- Microplate shaker

Reagent Preparation

- Bring all reagents to room temperature (16°C to 25°C) before use.

Diluent Solution – Ready to use as supplied.

Wash Solution Concentrate - Dilute 1:20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH₂O). 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 µL Detection Antibody to 990 µL 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 µL HRP-Streptavidin to 990 µL 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.

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

All biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

Calibrator preparation

The Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

1. Add 1.0 mL of distilled or de-ionized water to the Hamster (CHO) Phospholipase B-Like 2 stated on the vial.

Note: The reconstituted the Hamster (CHO) Phospholipase B-Like 2 (PLBL2) Calibrator should be aliquoted and stored frozen. Avoid multiple freeze-thaw cycles.

2. Label tube numbers 1 - 8.
3. Prepare Standard #1 by adding the appropriate volume of 1X Diluent Solution (see below) to tube #1. Add 50 µL of stock Hamster (CHO) Phospholipase B-Like 2 (PLBL2) Calibrator to obtain a concentration at 20 ng/mL and mix thoroughly and gently.

***Example: Note: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided.**

CS = Starting concentration of reconstituted Hamster (CHO) Phospholipase B-Like 2 (PLBL2) Calibrator

CF = Final concentration of Hamster (CHO) Phospholipase B-Like 2 (PLBL2) Calibrator for the assay procedure (20 ng/mL)

VA = Total volume of stock Hamster (CHO) Phospholipase B-Like 2 (PLBL2) Calibrator to dilute (e.g. 85 µL)

VD = Total volume of 1X Diluent Solution required to dilute stock Hamster (CHO) Phospholipase B-Like 2 (PLBL2) Calibrator to prepare Standard #1

VT = Total volume of Standard #1

DF = Dilution factor

Calculate the dilution factor (DF) between stock calibrator & the Standard #1 final conc.:

$$C_s/C_f = DF$$

$$240 / 20 = 12$$

Calculate the final volume VD required to prepare the Standard #1 at 20 ng/mL

$$V_A * DF = V_T$$

$$V_D = V_T - V_A$$

$$50 * 12 = 600 \mu\text{L}$$

$$V_D = 600 - 50 = 550 \mu\text{L}$$

To tube #1, add 50 μL of reconstituted Hamster (CHO) Phospholipase B-Like 2 (PLBL2) Calibrator to 550 μL of 1X Diluent Solution to obtain a concentration at 20 ng/mL (Standard #1).

4. Add 300 μL 1X Diluent Solution into tube numbers 2 - 8.
5. Prepare Standard #2 by adding 300 μL Standard #1 to tube #2. Mix thoroughly and gently.
6. Prepare Standard #3 by adding 300 μL from Standard #2 to #3. Mix thoroughly and gently.
7. Using the table below as a guide to prepare further serial dilutions. 1X Diluent Solution serves as the zero standard – Standard #8 (0 ng/mL)

Standard #	Volume to Dilute (μL)	Diluent (μL)	Total Volume (μL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See step 3				20
2	300	300	600	20	10
3	300	300	600	10	5
4	300	300	600	5	2.5
5	300	300	600	2.5	1.25
6	300	300	600	1.25	0.63
7	300	300	600	0.63	0.31
8	0	300	300	0	0

Sample Preparation

Sample Collection and Handling

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. Dilute samples immediately prior use.

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

Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
 - All standards and samples should be run at least in duplicate.
 - A standard curve should be run with each assay.
1. 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.
 2. Pipette 100 μL of Standards #1 -8.
 3. Pipette 100 μL of sample (in duplicate) into pre-designated wells.
 4. Incubate the micro titer plate while shaking on a microplate shaker at 400 rpm at room temperature for a hundred and twenty (120 ± 2) minutes. Keep plate covered and level during incubation.
 5. Following incubation, aspirate the contents of the well.
 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 μL of appropriately diluted Detection Antibody to each well. Incubate while shaking on a microplate shaker at 400rpm at room temperature for twenty (20 ± 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 μL of appropriately diluted HRP-streptavidin to each well. Incubate while shaking on a microplate shaker at 400rpm at room temperature for twenty (20 ± 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 μL of TMB Substrate Solution into each well.
 12. Incubate in the dark at room temperature for precisely ten (10) minutes.
 13. After ten minutes, add 100 μL 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.

Calculation

- Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
- 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.
- Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the PLBL2 concentration in original samples. Mix enough reagents for the number of assays to be performed, immediately before adding to the plate.

Technical Support

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