EAGLE BIOSCIENCES

Hamster (CHO) Lysosomal Phospholipase A2 (LPLA) ELISA Assay Kit

FOR REFERENCE USE ONLY

Catalog Number:
LPL91-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 4.1

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INTENDED USE

The Eagle Biosciences Hamster (CHO) Lysosomal Phospholipase A2 (LPLA) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of LPLA in CHO biological samples. The Eagle Biosciences Hamster (CHO) Lysosomal Phospholipase A2 (LPLA) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

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 or at 866-411-8023.

INTRODUCTION

Lysosomal phospholipase A (LPLA) is an enzyme that plays a crucial role in lipid metabolism by hydrolyzing lysosomal phospholipids into free fatty acids and glycerophosphocholine. In Chinese Hamster Ovary (CHO) cells—widely used in biopharmaceutical production for monoclonal antibodies and other recombinant proteins—LPLA has gained attention as a potential biomarker for cell health, productivity, and stress responses. LPLA is often localized to the lysosomes, and its expression or activity levels may reflect changes in lysosomal function, membrane turnover, or autophagic flux. Elevated or dysregulated LPLA activity in CHO cultures has been linked to cellular stress conditions, including nutrient depletion, accumulation of misfolded proteins, or process-related perturbations such as shifts in pH or osmolality.

In a research setting, LPLA is increasingly used to monitor bioprocess consistency and cell culture performance. For instance, real-time measurement of LPLA levels in cell culture supernatants or lysates can offer insights into cell viability or early apoptotic events, thereby informing optimization of feeding strategies or bioreactor parameters. Clinically, while CHO cells are not used in patients, LPLA's relevance extends to human health: elevated LPLA activity is associated with inflammatory diseases and lysosomal storage disorders, making it a translational biomarker in lysosome-targeted drug development. Additionally, leveraging CHO models expressing humanized versions of LPLA or using them to study LPLA-modulating drugs allows preclinical screening of candidate therapeutics. Thus, LPLA serves both as a diagnostic tool and a functional readout in drug discovery pipelines that utilize CHO platforms.

PRINCIPLE OF THE ASSAY

In this assay, the Lysosomal Phospholipase A2 (LPLA) present in samples reacts with the anti-LPLA antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, the Detection antibody, biotin conjugated anti-LPLA, 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 LPLA in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of LPLA in the test sample. The quantity of LPLA in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

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- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.3 sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.

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- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS
The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens, All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS
Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage
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.

Serum Samples: Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeat freeze-thaw cycles.

Plasma Samples: Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeat freeze-thaw cycles.

Urine Samples: Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeat freeze-thaw cycles.

**Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction and is a known interfering substance.*

Specimen Pre-Treatment
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.

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Lysate Samples: Recommended starting dilution is 1:40. To prepare a 1:40 dilution of a sample, transfer 20 µL of sample to 780 µL of 1x diluent. This gives you a 1:40 dilution.

Supernatant Samples: Recommended starting dilution is 1:10. To prepare a 1:10 dilution of a sample, transfer 50 µL of sample to 450 µL of 1x diluent. This gives you a 1:10 dilution. Mix thoroughly.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microplate washer/aspirator
- Distilled or Deionized H_20
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer
- Microplate shaker

REAGENTS PROVIDED

1. **Microplate**

Contents:	One antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label

2. **Detection Antibody (100x)**

Contents:	One bottle containing concentrated affinity purified antibody conjugated with biotin in a stabilizing buffer
Format:	Concentrated; Requires Preparation
Volume:	150 µL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label
Preparation of Working Solution:	**Dilute 1:100** in 1x diluent before use (e.g., 10 µL of antibody concentration in 990 µL of 1x diluent for each test strip). Dilute immediately before use and protect from light. Mix uniformly, but gently to avoid foaming.

3. **HRP Streptavidin Concentrate (100x)**

Contents:	One bottle containing concentrated horseradish peroxidase conjugated streptavidin in a stabilizing buffer.
Format:	Concentrated; Requires Preparation
Volume:	150 µL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label

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Preparation of Working Solution:	**Dilute 1:100** in 1x diluent before use (e.g., 10 µL of HRP concentration in 990 µL of 1x diluent for each test strip). Dilute immediately before use and protect from light. Mix uniformly, but gently to avoid foaming.

4. **Standard Concentrate**

Contents:	One bottle of lyophilized standard to be prepared for calculation of your standard curve.
Format:	Lyophilized, requires preparation
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Preparation: Use immediately, or aliquoted and frozen once reconstituted and not using entire plate.
Preparation of Working Solution:	Refer to the Certificate of Analysis (CofA). Working standard solution should be prepared immediately prior to use.

5. **Diluent Concentrate (5x)**

Contents:	One bottle containing a concentrated diluent buffer
Format:	Ready to Use
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Preparation: Stable for one week
Preparation of Working Solution:	**Dilute 1:5** with distilled or deionized water. 1 part concentrate with 4 parts water.

6. **TMB Substrate**

Contents:	One bottle containing 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide in citric acid buffer at pH 3.3
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C. Protect from light
Stability:	Stable until the expiry date printed on the label

7. **Stopping Solution**

Contents:	One bottle containing 0.3 M sulfuric acid.
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C
Stability	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

8. **Wash Buffer Concentrate (20x)**

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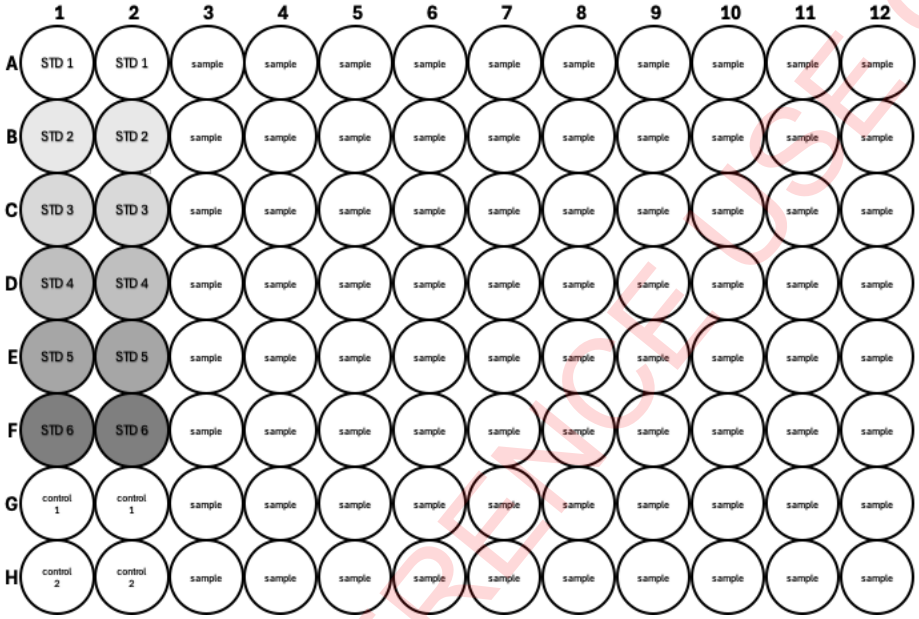
Contents:	One bottle containing a concentrated wash solution.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until expiry date printed on the label. After Preparation: Stable for one week.
Preparation of Working Solution:	**Dilute 1:20** in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 950 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT*



***** *Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Pipette 100 µL of standards and samples (in duplicate) into predesignated wells
2. Incubate the microplate on a shaker at 400 rpm at room temperature for 120 (±2) minutes. Keep plate covered and level during incubation
3. Following incubation, aspirate the contents of the wells.
4. 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.
5. Pipette 100 µL of appropriately diluted Detection Antibody to each well. Incubate while shaking on a plate shaker at 400 rpm at room temperature for twenty (20±2) minutes. Keep plate covered in the dark and level during incubation.

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6. Wash and blot the wells as described in Step 4
7. Pipette 100 µL of appropriately diluted HRP-streptavidin to each well. Incubate while shaking on a plate shaker at 400 rpm 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 Step 4
9. Pipette 100 µL TMB Substrate solution into each well
10. Incubate in the dark while shaking on a plate shaker at 400 rpm at room temperature for precisely ten (10) minutes.
11. After 10 minutes, add 100 µL of Stop Solution to each well
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specification.

CALCULATIONS

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 LPLA concentration in original samples.

QUALITY CONTROL

The test results are only valid if the test has been performed following the following instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated above and/or labeled. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation, the following technical issues should be reviewed. Expiration dates of prepared reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

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.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

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