LC3B ELISA Kit

LC3B ELISA Kit is an Enzyme Immunoassay kit for the quantification of LC3B in all samples.

Catalog number: ARG83364

Package: 96 wells

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INTRODUCTION

LC3B is an Ubiquitin-like modifier involved in formation of autophagosomal vacuoles (autophagosomes). Plays a role in mitophagy which contributes to regulate mitochondrial quantity and quality by eliminating the mitochondria to a basal level to fulfill cellular energy requirements and preventing excess ROS production. Whereas LC3s are involved in elongation of the phagophore membrane, the GABARAP/GATE-16 subfamily is essential for a later stage in autophagosome maturation. Promotes primary ciliogenesis by removing OFD1 from centriolar satellites via the autophagic pathway. Through its interaction with the reticulophagy receptor TEX264, paticipates in the remodeling of subdomains of the endoplasmic reticulum into autophagosomes upon nutrient stress, which then fuse with lysosomes for endoplasmic reticulum turnover.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for LC3B has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any LC3B present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for LC3B is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of LC3B bound in the initial step. The

color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of LC3B in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard at ≤ -80°C, 1000X LC3B Antibody at ≤ -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
Standard	1 vial (100 µL; 1 µg/mL)	≤ -80°C
Diluent Buffer	50 mL	4°C
100X LC3 Removal Reagent	1 mL	4°C
1000X LC3B Antibody	1 vial (50 µL)	≤ -20°C
1000X HRP-Streptavidin Solution	1 vial (20 µL)	4°C
10X Wash Buffer	100 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- 1X PBS (contain 1 mM $MgCl_2$ and 1 mM $CaCl_2$)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Standard at ≤ -80°C, 1000X LC3B Antibody at ≤ -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

REAGENT PREPARATION

- **100X LC3 Removal Reagent:** Dilute 100X LC3 Removal Reagent into 1X PBS (contain 1 mM MgCl2 and 1 mM CaCl2) to yield 1X LC3 Removal Reagent.
- **1X Wash Buffer:** Dilute **10X** Wash Buffer into distilled water to yield 1X Wash Buffer (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL).
- **1X LC3B Antibody:** 10 minutes before use, dilute **1000X** LC3B Antibody into Diluent Buffer to yield 1X Antibody Conjugate.
- **1X HRP-Streptavidin Solution:** 10 minutes before use, dilute **1000X** HRP-Streptavidin Solution into Diluent Buffer to yield 1X HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Standard:** Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. The Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Diluent Buffer. Diluted the standard as below:

Standard tube	LC3B (ng/mL)	Diluent Buffer (µL)	Standard (µL)
S1	20	490	10 (1 µg/mL Stock)
S2	10	250	250 of S1
S3	5	250	250 of S2
S4	2.5	250	250 of S3
S5	1.25	250	250 of S4
S0	0	250	0

Note: Working standard should be prepared immediately prior to use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

1. Culture cell in regular medium or starvation medium at 37°C for desired time to induce autophagy. (Agents that inhibit or stimulate autophagy can be added directly to the cell culture)
2. Wash cells with 1X PBS (contain 1 mM MgCl2 and 1 mM CaCl2).
3. Aspirate completely PBS and add 1.5 mL of 1X LC3 Removal Reagent. Incubate for 5 minutes at room temperature on shaker.
4. Aspirate completely 1X LC3 Removal Reagent, wash the cells 3 times with 1X PBS (contain 1 mM MgCl2 and 1 mM CaCl2).
5. Aspirate completely PBS, add lysis buffer to the cells.
6. Place the culture plates on ice for 10 minutes. Detach the cells from the plates by scraping with a cell scraper. Transfer the lysates to appropriate size tubes and place on ice.
7. Clear the lysates by centrifugation for 10 minutes (12,000 x g at 4°C)
8. Collect the supernatant, determine protein concentrations by BCA assay and store lysate samples on ice for immediate use, or store at-80°C for long-term storage.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

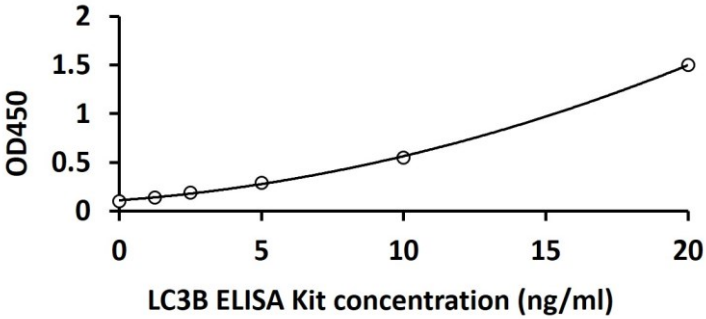
1. Add **100 µL** of **Samples, Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **2 hour** at **37˚C**.
3. Aspirate each well and wash, repeating the process 2 time for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X LC3B Antibody** to each well.
5. Cover the plate and incubate for **2 hour** at **room temperature**.
6. Aspirate each well and **wash** plate **as step 3**.
7. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
8. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
9. Aspirate each well and **wash** plate **as step 3**.
10. Add **100 µL** of **TMB Substrate** in each well.
11. Incubate for **5-30 mins** at **room temperature** in the dark.
12. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
13. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the LC3B ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.8 ng/ml

Assay Range

1.25 - 20 ng/ml