

Levetiracetam ELISA

For Research Use Only

Cat. No.: BW-LEV ELISA

1. Introduction

Levetiracetam ([*(S)*- α -ethyl-2-oxo-1-pyrrolidine acetamide], LEV) is an anticonvulsant drug available as a monotherapy for epilepsy in the case of partial seizures, or as an adjunctive therapy for myoclonic and tonic-clonic seizures.

LEV exhibits linear pharmacokinetics, minimal plasma protein binding, and low potential for drug–drug interactions, making it well-tolerated in most patients. However, therapeutic drug monitoring of levetiracetam is helpful in patients with conditions [1, 2, 3, 4, 5] that may alter pharmacokinetic characteristics (elderly, children, pregnancy, or renal impairment), to tailor individual dosage regimens or to investigate patient compliance.

This Levetiracetam ELISA is used for quantitative determination of levetiracetam in biological samples of epilepsy patients.

2. Principle of the Assay

The Levetiracetam ELISA adopts a typical competitive immunoassay principle.

Levetiracetam (LEV) in samples, calibrators, and controls competes with a LEV pre-coated on microplate wells for binding a limited number of anti-LEV monoclonal antibodies. Unbound components are removed by washing. Horseradish peroxidase (HRP)-conjugated secondary antibody is added to trace the bound primary antibody. After a final wash, TMB substrate is added to initiate a color reaction. The absorbance intensity is inversely proportional to the concentration of LEV in the sample. A calibration curve is established using calibrators to calculate the LEV concentration in unknown samples.

3. Package Contents

Kit contents are sufficient for 96 tests (8×12 wells).

Expiry date is labeled on the outer box.

Components	Specification	Description
① LEV Coated Plate	8×12 wells, detachable	Microwells pre-coated with LEV
② Primary Antibody	1 bottle (0.5mL, 25×)	Monoclonal antibody against LEV
③ Assay Buffer	1 bottle (30mL)	PBS buffer containing BSA and Tween-20
④ Secondary Antibody	1 bottle (0.5mL, 25×)	Sheep anti-mouse IgG- HRP conjugate
⑤ TMB Chromogenic Substrate	1 bottle (15mL)	Single-component TMB for color development
⑥ Wash Buffer	2 bottles (25mL/bottle, 10×)	0.05% Tween-20 in PBS
⑦ Stop Solution	1 bottle (15mL)	1 M Citric acid solution
⑧ LEV Calibrators	6 vials (1mL each)	0, 2, 8, 20, 50, 100µg/mL

4. Kit Storage and Stability

- 1) Store the entire kit at 2–8°C.
- 2) Unused plate strips must be kept in the aluminum foil bag with desiccant; keep sealed and dry.

- 3) Shelf life: 12 months from production.
- 4) Use opened reagents and plate strips within 3 months.

5. Equipment and Materials Required but not Provided

- 1) Single-channel micropipettes (10–100 μ L) and disposable tips
- 2) 8/12-channel micropipette (50–200 μ L)
- 3) Orbital microplate shaker
- 4) Microplate reader (detection wavelength: 450nm)
- 5) Distilled or deionized water
- 6) Microplate washer (optional)

6. Sample Collection and Storage

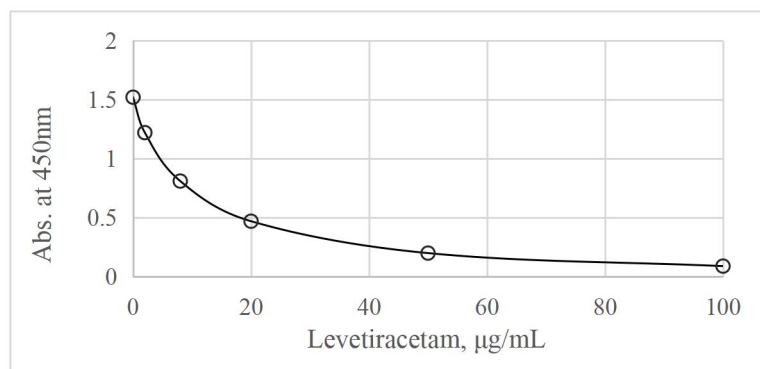
- 1) The test can be performed with serum and plasma as samples. The applicability for testing samples other than plasma or serum remains to be verified.
- 2) Hemolytic and lipemic samples should not be used.
- 3) The samples can be stored at 2 ~ 8°C up to one month with < 5% reduction in concentration. For a longer storage (> 1 months) the samples need to be frozen at -20 ~ -80°C. Repeated freezing and thawing should be avoided.
- 4) Hemolytic, lipemic and icteric specimens should not be used.
- 5) Handle all patient samples as if they were potentially infectious.

7. Experimental Procedure

- 1) Separate the strips to be used by cutting through the protective tape with a sharp knife and transfer them to a strip frame. Pull off the protective tape from the strips intended for use. Return the remaining strips to the plastic tray pack and reseal.
- 2) According to the number of samples to be tested, add the Primary Antibody to the required volume of Assay Buffer at a ratio of 1:25, and mix thoroughly. This should be done within 60 minutes before use.
- 3) Pipette 25 μ L of the LEV calibrators and samples into the bottom of each well.
- 4) Add 75 μ L of diluted Primary Antibody solution to each well, incubate the frame for 90 minutes at room temperature on an orbital shaker at medium speed.
- 5) Discard or aspirate the contents of the wells and wash thoroughly with 300 μ L wash buffer diluted 10 times with distilled water, remove residual liquid by tapping the inverted plate on a clean absorbent paper. Repeat this 3 times. Preferably, a Microplate Washer may be used.
- 6) Within 60 minutes before use, prepare the needed volume of Secondary Antibody solution by mixing 1.5mL Assay Buffer with 60 μ L Secondary Antibody stock solution (25 $\times$) per strip.
- 7) Dispense 100 μ L of diluted Secondary Antibody solution into each well and incubate the frame for 60 minutes at room temperature on an orbital shaker at medium speed.
- 8) After incubation, wash the wells as in step 7-5). Ensure no residual wash buffer remains.
- 9) Dispense 100 μ L of Chromogenic Substrate solution into each well, and mix thoroughly. Incubate for 20 minutes at room temperature with slow shaking.
- 10) Add 100 μ L of Stop Solution into each well. Shake for at least 10 seconds on an orbital shaker.
- 11) Quantitation. Read the optical density at 450nm in a microplate photometer within 10 minutes. Plot absorbance vs. calibrator concentration to construct the LEV calibrator curve. When using analyzing software, it is recommended to use the Four Parameter Logistic (4PL) Regression. The concentration of the controls and samples in ng/ml can be read directly from this calibration curve by using their average optical density.

8. Performance Characteristics

- 1) The typical standard curve for the LEV-ELISA is shown below.



- 2) Analytical sensitivity. The analytical sensitivity of the LEV-ELISA is typically better than 0.5 µg/mL, defined as the mean of the measurement values for the zero calibrator minus 2 standard deviations (mean value - 2SD).
- 3) Recovery. Spiked serum samples were prepared by adding varying levels of LEV to pooled serum specimens containing a known amount of LEV. Recoveries were in the range of 93- 102% with a mean value of 96.6% (n=15).
- 4) The cross-reactivity (at the 50% displacement level) with the major inactive metabolites of LEV, UCB L057, was < 0.26%.
- 5) Intra- assay precision: ≤ 10%, Inter- assay precision: ≤ 15%.
- 6) The range of measurement: 2- 100µg/mL.

9. Notes

- 1) This kit is for research use only, not for human diagnostic or therapeutic procedures.
- 2) Avoid repeated freezing and thawing of reagents.
- 3) Protect TMB substrate from light and avoid contact with metal ions.
- 4) Strictly follow incubation time and temperature to ensure reproducibility.
- 5) If the OD value of the sample exceeds the range of the calibration curve, the sample needs to be diluted with the zero-concentration calibrator for detection to ensure that the result is within the linear range.

10. References

- 1) Yoshiaki Yamamotoa, Akiko Ohtaa, Naotaka Usui Bleyzac N, et al. Clinical value of therapeutic drug monitoring for levetiracetam in pediatric patients with epilepsy. *Brain & Development* 45 (2023) 285–292.
- 2) Noam Fallik, Ilia Trakhtenbroit, Firas Fahoum, et al. Therapeutic drug monitoring in pregnancy: Levetiracetam. *Epilepsia*. 2024;65:1285–1293.
- 3) Hoffer E, Akria L, Tabak A, et al. A simple approximation for LEV dose adjustment in adult patients undergoing bone marrow transplantation. *Ther Drug Monit*. 2004, 26, 331–335.
- 4) Eduard Valdes, Taolin Fang, Michael Boffa, et al. Optimal Dosing of Levetiracetam for Seizure Prophylaxis in Critically Ill Patients: A Prospective Observational Study. *Critical Care Medicine*, 2024, 52, e1-e10.
- 5) Min Shi, Chenxi Liu, Lien He, et al Therapeutic drug monitoring and the therapeutic reference range of levetiracetam for Chinese patients: Problems and issues. *Seizure: European Journal of Epilepsy*. 2023, 109, 26-33.

Developed and Manufactured by:

Shanghai Baiwei Biotechnology Co., Ltd.

Address: Unit C401-3, Building 7, Pujiang Wisdom Valley, No.1188 Lianhang Road, Minhang District, Shanghai, China.

URL: bowei-biotech.com

Contact: +8615221067868

