Bowei-biotech
for better bioassays

Venetoclax ELISA

For Research Use Only
Cat. No.: BW-Venetoclax ELISA

1. Introduction

Venetoclax is a highly selective B-cell lymphoma-2 (BCL-2) inhibitor used in the treatment of chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), acute myeloid leukemia (AML), and other hematologic malignancies. Due to its high selectivity for BCL-2 protein, venetoclax shows superior efficacy and better safety compared to other group of drugs [1, 2]. However, when venetoclax is administered at a fixed dose, the plasma concentration of venetoclax will vary widely between individuals, especially when venetoclax is co-administered with a strong CYP3A4 inhibitor. The continued use of venetoclax at high doses can result in life-threatening tumor lysis syndrome (TLS, 12%), and some frequent adverse events including neutropenia (45%), diarrhea (35%), nausea (33%), anemia (29%), and thrombocytopenia (22%) [3]. Close monitoring of the blood venetoclax concentration is highly recommended with an aim to reduce the risk of tumor-lysis syndrome and other complications including severe neutropenia and thrombocytopenia.

This Venetoclax ELISA is designed for the quantitative determination of venetoclax in serum or plasma.

2. Principle of the Assay

The Venetoclax ELISA adopts a typical competitive immunoassay principle.

Venetoclax in samples, calibrators, and controls competes with the venetoclax pre-coated on microplate wells for binding a limited number of rabbit anti-venetoclax polyclonal 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 venetoclax in the sample. A calibration curve is established using calibrators to calculate the venetoclax 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 Venetoclax Coated Plate	8×12 wells, detachable	Microwells pre-coated with venetoclax
② Primary Antibody	1 bottle (0.5mL, 25×)	Rabbit antibody against venetoclax
③ Assay Buffer	1 bottle (30mL)	PBS buffer containing BSA and Tween-20
④ Secondary Antibody	1 bottle (0.5mL, 25×)	Sheep anti-rabbit 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 Venetoclax Calibrators	6 vials (1mL each)	0, 0.2, 0.5, 1.0, 2.5, 5.0μg/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 (10–100µL) and disposable tips
2) 8/12- channel micropipette (50–200µL)
3) Orbital microplate shaker
4) Microplate reader (detection wavelength: 450nm)
5) Distilled or deionized water
6) Microplate washer (optional)

6. Sample Collection and Storage

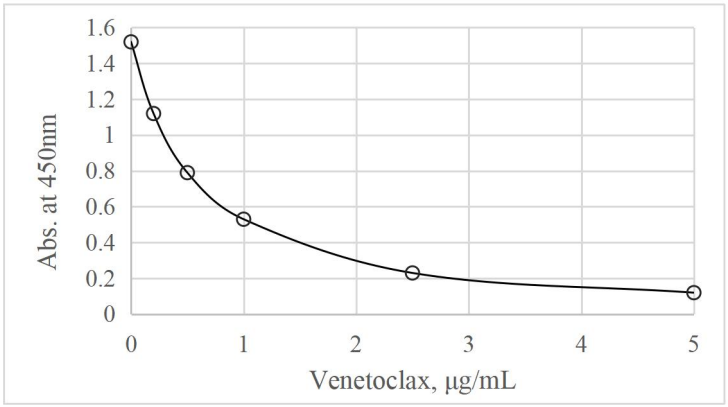
1) The test can be performed with serum and plasma as samples. The applicability for testing samples other than plasma or serum remains to be verified.
2) Hemolytic and lipemic samples should not be used.
3) Venetoclax in samples is stable up to 3 months at 2 -8°C with < 5% reduction in concentration. For a longer storage (> 3 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 25µL of the Venetoclax calibrators and samples into the bottom of each well.
4) Add 75µL 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 300µl 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 60µL Secondary Antibody stock solution (25×) per strip.
7) Dispense 100µL 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 100µL of Chromogenic Substrate solution into each well, and mix thoroughly. Incubate for 20 minutes at room temperature with slow shaking.
10) Add 100µL 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 venetoclax 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 µg/ml can be read directly from this calibration curve by using their average optical density.

8. Performance Characteristics

1) The typical calibration curve for the Venetoclax-ELISA is shown below.



2) Analytical sensitivity. The analytical sensitivity of the Venetoclax-ELISA is typically better than 0.06μg/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 Venetoclax to pooled serum specimens containing a known amount of Venetoclax. Recoveries were in the range of 90.8- 103.2% with a mean value of 95.7% (n=15).
4) The cross reactivity (at the 50% displacement level) with M27, the major metabolites of venetoclax, was < 0.03%.
5) Intra- assay precision: ≤ 10%, Inter- assay precision: ≤ 15%.
6) The range of measurement: 0.1- 5μg/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) Konopleva M, Pollyea DA. Potluri J, et al. Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia. Cancer Discov. 2016, 6, 1106–1117.
2) Eyre TA, Roeker LE, Fox CP, et al. The efficacy and safety of venetoclax therapy in elderly patients with relapsed, refractory chronic lymphocytic leukaemia. Br. J. Haematol. 2020, 188, 918–923.
3) Al-Sawaf O, Zhang C, Tandon M, et al. Venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab for previously untreated chronic lymphocytic leukaemia (CLL14): Follow-up results from a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2020, 21, 1188–1200.

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