Bowei-biotech
for better bioassays

Busulfan ELISA

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

1. Introduction

Busulfan is a commonly used alkylating agent in conditioning regimens before hematopoietic stem cell transplantation (HSCT) and for the treatment of myeloproliferative neoplasms [1].

Busulfan has a narrow therapeutic exposure window, which varies with disease type and graft sources. Elevated blood busulfan concentrations are proven to raise the risk of severe hepatic veno-occlusive disease, an intractable condition with no effective treatment. By contrast, suboptimal low busulfan levels are linked to the relapse of chronic myeloid leukemia, and further reduced exposure may lead to graft rejection. Therapeutic drug monitoring (TDM) of busulfan is therefore essential for balancing therapeutic efficacy and adverse toxicity, thereby enhances the success rates of HSCT and improves clinical patient outcomes. A therapeutic range for busulfan in HSCT is generally accepted as an AUC from 800 to 1500μmol·min/L [2, 3, 4, 5].

This Busulfan ELISA is designed for rapid and convenient monitoring of busulfan levels in serum or plasma samples.

2. Principle of the Assay

The Busulfan ELISA adopts a typical competitive immunoassay principle.

Busulfan in samples, calibrators, and controls competes with a busulfan analogue pre-coated on microplate wells for binding a limited number of anti-busulfan 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 busulfan in the sample. A calibration curve is established using calibrators to calculate the busulfan 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 Busulfan Coated Plate	8×12 wells, detachable	Microwells pre-coated with busulfan analogue
② Primary Antibody	1 bottle (0.5mL, 25×)	Monoclonal antibody against busulfan
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 Busulfan Calibrators	6 vials (1mL each)	0, 100, 250, 500, 1000, 2000ng/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

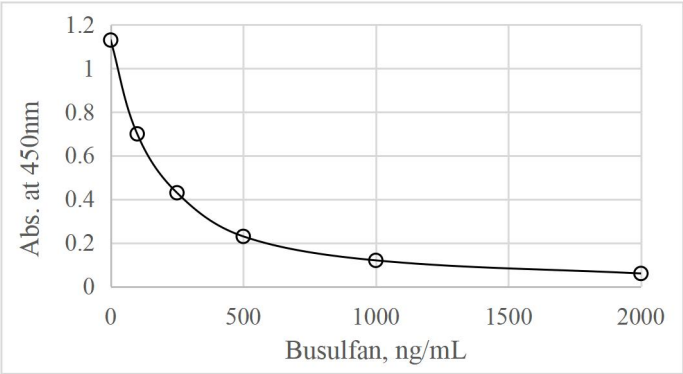
1) The blood should be centrifuged within 3h of collection. The test can be performed with serum and plasma as samples. The applicability for testing samples other than plasma or serum remains to be verified.
2) Hemolytic and lipemic samples should not be used.
3) The samples can be placed on an ice surface (0 ~ 3°C) up to 24h with < 5% reduction in concentration and AUC. 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 Busulfan 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 Busulfan 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 Busulfan-ELISA is shown below.



2) Analytical sensitivity. The analytical sensitivity of the Busulfan-ELISA is typically better than 26ng/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 Busulfan to pooled serum specimens containing a known amount of Busulfan. Recoveries were in the range of 96- 112% with a mean value of 97.5% (n=15).
4) The cross reactivities (at the 50% displacement level) with the major inactive metabolites of Busulfan were listed below.

Substance	Cross Reactivity (%)
Busulfan	100
Tetrahydrothiophene, , and	< 0.01%
tetramethyl sulfone	< 0.01%
tetrahydrothiophene-3-ol-1,1-dioxide	< 0.01%

5) Intra- assay precision: ≤ 10%, Inter- assay precision: ≤ 15%.
6) The range of measurement: 0.1- 10ng/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) Vera Domingos, Katerina Nezvalova-Henriksen, Adrin Dadkhah, et al. A practical guide to therapeutic drug monitoring in busulfan: recommendations from the Pharmacist Committee of the European Society for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplantation, 2024, 59:1641–1653. https://doi.org/10.1038/s41409-024-02413-0.

2) Bleyzac N, Souillet G, Magron P, et al. Improved clinical outcome of paediatric bone marrow recipients using a test dose and Bayesian pharmacokinetic individualization of busulfan dosage regimens. Bone Marrow Transplant. 2001, 28, 743–751.

3) Hoffer E, Akria L, Tabak A, et al. A simple approximation for busulfan dose adjustment in adult patients undergoing bone marrow transplantation. Ther Drug Monit. 2004, 26, 331–335.

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