Version 23-04/24

User's Manual

Human β-lactamase ELISA Kit

REF **DEIANJ17**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This ELISA kit is used for quantitative determination of β-lactamase.

General Description

Extended spectrum beta-lactamase (ESBL) that inactivates beta-lactam antibiotics by hydrolyzing the amide group of the beta-lactam ring. Displays high levels of penicillinase and cephalosporinase activity as well as measurable activity with carbapenems, including imipenem and meropenem. Plays a primary role in the intrinsic resistance of M.tuberculosis to beta-lactam antibiotics. B

Principles of Testing

β-lactamase ELISA kit is a sandwich ELISA assay for the quantitative measurement of human β-lactamase in serum, plasma and cell culture supernatants. The density of color is proportional to the amount of human β-lactamase captured from the samples.

Reagents And Materials Provided

Micro ELISA strip-plate 1

Standard (5400 pg/ml) 0.5 ml

Standard diluent 1.5 ml

HRP- Conjugate reagent 6 ml

Sample diluent 6 ml

Chromogen Solution A 6 ml

Chromogen Solution B 6 ml

Stop Solution 6 ml

Wash buffer (30×) 20 ml

Plate sealers 2

Materials Required But Not Supplied

Microplate reader capable of measuring absorbance at 450 nm

37° C incubator

Precision pipettes with disposable tips

Distilled or deionized water

Clean eppendorf tubes for preparing standards or sample dilutions

Absorbent paper

Storage

The entire kit may be stored at 4°C in dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

Specimen Collection And Preparation

Note: Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay can not be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with NaN3 should be avoided for this assay.

Serum: After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuge again.

Plasma: Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuge again.

Urine: Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

Cell Samples: If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1×100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again.

Tissue Samples: Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4°C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

Reagent Preparation

Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. Wash Buffer: Dilute the concentrated washing buffer (30×) with distilled water.
2. Standard Preparation:

Ten wells are set for standards in a ELISA-plate. In Well 1 and Well 2, 100 µl Standard solution and 50 µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 100 µl solution from Well 1 and Well

2 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 9 and Well 10.

Suggested standard concentration: 3600 pg/ml, 2400 pg/ml,1200 pg/ml, 600 pg/ml and 300 pg/ml

Assay Procedure

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

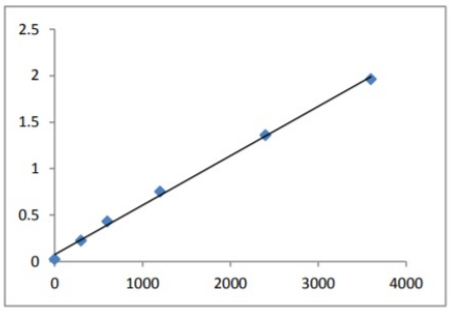
1. Prepare all reagents, samples and standards as instructed before.
2. In sample wells, add 40 µl Sample dilution buffer and 10 µl samples are added (dilution factor is 5). Leave a well empty as blank control. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubate 30 min at 37°C after sealed with Closure plate membrane.
4. Remove plate sealer, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
5. Add 50 µl HRP-Conjugate reagent to each well except the blank control well. Incubate 30 min at 37°C.
6. Washing as described in Step 4.
7. Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes. Please avoid light during coloring.
8. Add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
9. Read absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.

Calculation

Known concentrations of Human β-lactamase Standard and its corresponding reading OD is plotted respectively. The concentration of Human β-lactamase in sample is determined by plotting the sample's O.D. on the X-axis. The original concentration is calculated by multiplying the dilution factor.

Typical Standard Curve

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



Detection Range

300 - 3600 pg/ml

Sensitivity

< 22 pg/ml