Version 11-04/24

User's Manual

Doxorubicin ADC ELISA Kit

REF **DEIABL322**

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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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This highly sensitive "sandwich" test kit is intended for use in the quantitative determination of antibody doxorubicin conjugate level in serum of human. It is useful for pre-clinical and clinical pharmacology study of doxorubicin Antibody Drug Conjugate (ADC).

Principles of Testing

This ELISA kit is designed, developed and produced for the quantitative measurement of antibody doxorubicin conjugate in serum. The assay utilizes the sandwich immunoassay technique with an antibody that binds to doxorubicin. Briefly, Anti-doxorubicin antibody is coated onto a microtiter plate. In the assay system, the assay calibrators, controls and test specimen are added to this microtiter plate. During the first incubation period, the anti-doxorubicin antibody captures the Doxorubicin-Antibody Conjugate of calibrators, controls and test samples. Unbound proteins are washed away with a wash step. A HRP (horseradish peroxidase) conjugate anti-human IgG tracer antibody is added to each well of the microtiter plate. After the second incubation, a "sandwich" immunocomplex of "Anti-doxorubicin antibody - doxorubicin Antibody Conjugate - HRP-conjugated anti-human IgG antibody" is formed and attached to the wall of the plate. The unbound HRP-conjugated antibody is removed in a subsequent washing step. For the detection of this immunocomplex, each well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to doxorubicin Antibody Conjugate on the wall of the microtiter well is directly proportional to the amount of doxorubicin Antibody Conjugate level in the sample.

Reagents And Materials Provided

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. **Anti-Doxorubicin Antibody Coated Microplate**

   One microplate with twelve by eight strips (96 wells total) coated with Anti-doxorubicin antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 - 8 °C and is stable until the expiration date on the kit box.

2. **Doxorubicin Tracer Antibody**

   One vial containing 12mL of ready to use Doxorubicin Tracer Antibody in a stabilized protein matrix. This reagent should be stored at 2-8°C and is stable until the expiration date on the kit box.

3. **ELISA Wash Concentrate**

   One bottle containing 50 mL of 20-fold concentrate. Before use the contents must be diluted with 950 mL of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

4. **ELISA HRP Substrate**

   Two bottles containing 6 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should

be stored at 2 - 8°C and is stable until the expiration date on the kit box.

5. **ELISA Stop Solution**

   One bottle containing 12 mL of stop solution. This reagent may be stored at 2 - 8°C or room temperature and is stable until the expiration date on the kit box.

6. **Assay Buffer**

   One bottle containing 12 mL ready-to-use buffer. It should be used according to the assay procedures. This reagent should be stored at 2 - 8°C and is stable until the expiration date on the kit box.

7. **Antibody Conjugated Calibrator Zero**

   One vial containing 30 mL calibrator zero. This reagent is used for diluting the calibrator stock to make assay calibrators, as well as for diluting test samples. This reagent should be stored at 2-8°C and is stable until the expiration date on the kit box.

8. **Antibody-Doxorubicin Conjugated Calibrator Stock**

   One vial containing 50µL of 100µg/mL antibody doxorubicin conjugated calibrator stock in a serum-based matrix. Refer to the vial for exact concentration of the calibrator. This calibrator should be stored at 2 - 8°C and is stable until the expiration date on the kit box.

Materials Required But Not Supplied

1. Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, etc.
2. Disposable pipette tips suitable for above volume dispensing.
3. Aluminum foil.
4. Deionized or distilled water.
5. Plastic microtiter well cover or polyethylene film.
6. ELISA multichannel wash bottle or automatic (semiautomatic) washing system.
7. Spectrophotometric microplate reader capable of reading absorbance at 450/620 nm.

Storage

This test kit must be stored at 2°C~8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Specimen Collection And Preparation

Serum is a suitable specimen for Doxorubicin-ADC measurement. Only 10 µL of samples is required for a duplicate determination of Doxorubicin-ADC with this test kit. No special preparation of individual is necessary prior to specimen collection. Samples should be collected by standard technologies of clinical laboratory practice and recommended by manufacturer of sample collection tube. Samples should be transferred to a clean test tube right after centrifugation and should be stored at 2 - 8°C if the assay is to be performed within 72 hours. Otherwise, samples should be stored at -20°C or below until measurement. Avoid more than three times freeze-thaw cycles of specimen. Do not use hemolyzed, hyperlipermic, heat-treated or any contaminated specimens.

Reagent Preparation

1. Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior to use.
3. Using CD Calibrator Stock: Dilute the reconstituted calibration stock to 1:X* using the zero calibrator and then diluted to obtain a level six calibrator at 390 ng/mL. Further create calibrator level five to two by 1:3 serialdilutions to obtain these calibrators with concentrations of 130 ng/mL,43.3 ng/mL,14.4 ng/mL, 4.8 ng/mL.

   Assay calibrators should be used within 24 hours and should be stored below -20°C. Do not exceed 3 freeze-thaw cycles.

   X* = the concentration of calibration stock / 390

   The validation data of this test was generated by using CD Antibody-Doxorubicin Conjugated Calibrator Stock.
4. When using own doxorubicin calibrator stock, the user may follow step3 (using CD calibrator stock) as a reference. Every ADC is created with different DARs and conjugation methods. It is recommended to make in- house calibration curve.
5. Each unknown sample needs to be diluted 1:100 using Antibody Conjugated Calibrator Zero.

Assay Procedure

Notes:

1. It is recommended that all standards and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results. It is recommended to add external controls to each assay.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. An orbital mixer with a larger orbit radius (e.g.> 1 cm) may be used at speeds of 400 to 450 rpm.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
9. If adapting this assay to automated ELISA system such as DS-2, DSX or Trituras, a procedural validation is necessary if there is any modification of the assay procedure.

Procedure

1. Add 25 µL of calibrators and diluted 1:100 test samples into the designated microwells. Tap the plate gently.
2. Immediately add 100 µL of Assay Buffer.
3. Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 1 hour at 400 to 450 rpm.

4. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
5. Add 100 µL of Doxorubicin Tracer Antibody to each well. Tap the plate gently.
6. Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 30 minutes at 400 to 450 rpm.
7. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
8. Add 100 µL of ELISA HRP Substrate into each of the wells.
9. Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate plate static, at room temperature for 20 minutes.
10. Immediately add 100 µL of ELISA Stop Solution into each of the wells. Mix gently.
11. Read the absorbance at 450 nm with reference filter at 620 nm.

Quality Control

To assure the validity of the results each assay should include adequate controls.

Calculation

It is recommended to use the four-parameter standard curve fitting.

1. Calculate the average absorbance for each pair of duplicate test results.
2. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration. Appropriate computer assisted data reduction programs should be used for the calculation of results.

The antibody-doxorubicin conjugate concentrations for the test samples are read directly from the standard curve using their respective corrected absorbance.

Typical Standard Curve

A typical absorbance data and the resulting standard curve from this Doxorubicin ADC ELISA are represented. This curve should not be used in lieu of standard curve generated with each assay.

Precision

Intra-assay Precision (Precision within an assay): ≤10%. Inter-assay Precision (Precision between assays) : ≤15%.

Detection Range

0-390ng/mL

Sensitivity

The limit of quantitation(LOQ) was 30ng/mL, the concentration corresponding to the value obtained by test antibody doxorubicin conjugate spiked to a low concentration of 30ng/mL under the precision CV<15% and the recovery in the range of 80-120%.

Recovery

The recovery of antibody doxorubicin conjugate spiked to different levels throughout the range of the assay in related matrices was evaluated.

Precautions

The reagents must be used in professional laboratory. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes.

Use Good Laboratory Practices.

Limitations

1. This assay requires serum sample for testing. Cell culture or tissue culture samples not yet validate.
2. For sample values greater than 390 µg/mL, it is recommended to re-assay samples with further dilution with calibrator zero.
3. The kit standards are based on doxorubicin conjugated antibody or ADC concentration. It is not based on free doxorubicin concentration. The doxorubicin-ADC in different linker and DAR may give different curve shift.
4. If a higher analytical test sensitivity is desired, a modification by increasing the test sample volume from 25 µl per well to 50 µl or 100 µl per well along with a longer first incubation time period would be very helpful. Please call for technical support.