Version 27-04/24

User's Manual

DM1 ADC ELISA Kit

REF **DEIABL311**

Σ **96T**

RUO

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.

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PRODUCT INFORMATION

Intended Use

This highly sensitive "sandwich" test kit is intended for use in the quantitative determination of antibody DM1 conjugate level in human serum or plasma. It is useful for pre-clinical and clinical pharmacology study of DM1 Antibody Drug Conjugate (ADC).

Principles of Testing

This ELISA kit is designed, developed and produced for the quantitative measurement of antibody DM1 conjugate in serum or plasma. The assay utilizes the sandwich immunoassay technique with an antibody that binds to DM1. Briefly, Anti-DM1 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-DM1 antibody captures the DM1-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-DM1 antibody – DM1 Antibody Drug 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 DM1 Antibody Conjugate on the wall of the microtiter well is directly proportional to the amount of DM1 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-DM1 Antibody Coated Microplate.** One microplate with twelve by eight strips (96 wells total) coated with Anti-DM1 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. **DM1 Tracer Antibody.** One vial containing 12mL of ready to use DM1 Tracer Antibody in a stabilized protein matrix. This reagent should be stored at 2-8°C and is table 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-DM1 Conjugated Calibrator Stock-Not provided in the kit (optional).** One vial containing the calibration stock of antibody DM1-conjugate which is Trastuzumab emtansine. The DAR is 3.5. Refer to the vial for exact concentration of the standard. This reagent should be stored at 2-8°C, sealed storage, away from moisture and light.

* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)

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 samples are suitable specimens for DM1-ADC measurement. Only 10 µL of samples is required for a duplicate determination of DM1-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, patient 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 seven calibrator at 500 ng/mL. Further create calibrator level six to two by 1:2 serial dilutions to obtain these calibrators with concentrations of 200 ng/mL, 100 ng/mL, 50 ng/mL, 25ng/mL,

12.5ng/mL, 6.25ng/mL, 3.125ng/mL. Assay calibrators should be used within 24 hours and should be stored below -20°C. Do not exceed 3 freeze-thaw cycles.

Example of making collection 1-7:			
Calibrator	Calibrator Volume	Dilution Factor	Volume of Cal0
Stock-1mg/mL*	Refer to the label for exact stock concentration		
Cal7 200ng/mL	1μL of stock into 1mL Cal0 to get 1μg/mL and then pipette 200μL of 1μg/mL to 800μL Cal0 to get 200ng/mL		
Cal6 100ng/mL	0.5mL of Cal7	1:2	0.5mL
Cal5 50ng/mL	0.5mL of Cal6	1:2	0.5mL
Cal4 25ng/mL	0.5mL of Cal5	1:2	0.5mL
Cal3 12.5ng/mL	0.5mL of Cal4	1:2	0.5mL
Cal2 6.25ng/mL	0.5mL of Cal3	1:2	0.5mL
Cal1 3.125ng/mL	0.5mL of Cal2	1:2	0.5mL
Cal0 0ng/mL	-	-	-

The validation data of this test was generated by using CD Antibody-DM1 Conjugated Calibrator Stock.

4. When using own DM1 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 about 250 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 about 250 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 DM1 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 about 250 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 4-parameter logistics curve-fitting algorithm. Laboratories can also establish other applicable fitting methods, like quadratic curve regression.

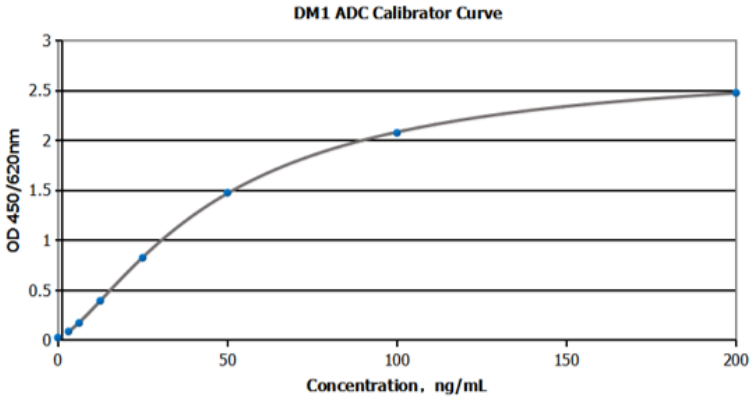
1. Calculate the average absorbance for each pair of duplicate test results.
2. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration. Appropriate computer assisted data reduction programs should be used for the calculation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

The antibody-DM1 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 DM1 ADC ELISA are represented. This curve should not be used in lieu of standard curve generated with each assay.

Well I.D.	OD450/620nm Absorbance		
	Reading		Average
200ng/mL	2.4869	2.4591	2.473
100ng/mL	2.0672	2.0767	2.072
50ng/mL	1.4659	1.4791	1.473
25ng/mL	0.8278	0.8153	0.822
12.5ng/mL	0.385	0.3957	0.390
6.25ng/mL	0.1619	0.1744	0.168
3.125ng/mL	0.0843	0.0824	0.083
0ng/mL	0.0226	0.022	0.022



Precision

Intra-assay Precision (Precision within an assay):2.10%.

Inter-assay Precision (Precision between assays) :7.36%.

Sensitivity

The limit of quantitation(LOQ) was 10ng/mL, the concentration corresponding to the value obtained by test DM1 Antibody Drug Conjugate (ADC) spiked to a low concentration of 10ng/mL under the precision CV < 15% and the recovery in the range of 80-120%.

Hook:250ng/mL.

Specificity

This DM1-ADC ELISA doesn't show any cross reactivity to MMAE-ADC or SN38-ADC.

Recovery

The recovery of DM1 Antibody Drug Conjugate (ADC) spiked to different levels throughout the range of the assay in related matrices was evaluated.

Recovery		
Serum	200ng/mL	93%
	100ng/mL	100%
	10ng/mL	114%
Plasma	200ng/mL	96%
	100ng/mL	106%
	10ng/mL	86%

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.

Limitations

1. This assay requires serum or plasma sample for testing. Cell culture or tissue culture samples not yet validate.
2. For sample values greater than 200 ng/mL, it is recommended to re-assay samples with further dilution with calibrator zero.
3. The kit standards are based on DM1 conjugated antibody or ADC concentration. It is not based on free DM1 concentration. The DM1-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.