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EDI™ MMAE ADC EIA Kit

Enzyme Immunoassay (EIA) for the Quantitative Measurement of Antibody-MMAE-Conjugate Level in Serum (human, mouse, rat, primate, etc.)

REF **KTR-757** Σ 96 +2 +8 °C

For Research Use Only Not for Use in Diagnostic Procedures

I. INTENDED USE

This test kit is intended for use in the quantitative determination of antibody-MMAE-conjugate level in test sample. It is useful for pre-clinical and clinical pharmacology study of MMAE Antibody Drug Conjugate (ADC).

- Samples from tissue/cell culture and serum samples from human, rat, mouse, primate, etc. can be used directly with this kit.
- Both humanized monoclonal antibody based MMAE-ADC and mouse monoclonal antibody based MMAE-ADC can be measured with this kit.

II. ASSAY PRINCIPLE

This EIA kit is designed, developed and produced for the quantitative measurement of antibody MMAE conjugate in serum. The assay utilizes the competitive immunoassay technique with an antibody that exclusively binds to MMAE.

Assay calibrators (antibody MMAE conjugate) and test serum samples are added directly to wells of a microtiter plate that is coated with specific anti-MMAE antibody. Subsequently, a horseradish peroxidase (HRP) conjugated MMAE is added to each well. During the incubation period, the antibody MMAE conjugate competes with the HRP conjugated MMAE for the limited binding sites of anti-MMAE antibody. An immune complex of well coated "anti-MMAE antibody – HRP conjugated MMAE" is formed. The unbound antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction, which is terminated with an acidic reagent (i.e. ELISA stop solution). The absorbance is then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is inversely proportional to the amount of antibody-MMAE conjugate in the test sample. A calibration curve is generated by plotting the absorbance versus the respective antibody-MMAE conjugate concentration for each calibrator on a 4-parameter or log-logit curve fitting. The concentration of antibody-MMAE conjugate in test samples is determined directly from this calibration curve.

III. REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 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.

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-MMAE Antibody Coated Microplate (Cat. No. 30748)
One microplate with twelve by eight strips (96 wells total) coated with specific anti-MMAE 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. HRP Conjugated MMAE (Cat. No. 30685)
One vial containing **3 mL** of ready to use HRP labeled MMAE 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 (Cat. No. 10010)
One bottle containing **30 mL** of 30-fold concentrate. Before use the contents must be diluted with **870 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 (Cat. No. 10020)
One bottle containing **15 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 (Cat. No. 10030)
One bottle containing **15 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. Antibody-MMAE Conjugated Zero Calibrator (Cat. No. 30711)
One vial containing **30mL** zero calibrator (30711). This reagent is used for diluting the calibration stock t make assay calibrators, as well as for diluting test samples. This reagent should be stored at 2-8ºC and is stable until the expiration date on the kit box.

7. Antibody-MMAE Conjugated Calibration Stock (Cat. No. 30712) – *Not provided in the kit (optional)*
One vial (30712) containing the calibration stock of antibody-MMAE-conjugate in a lyophilized (**0.5 mL**) serum based matrix with a non-azide preservative. **Refer to the vial for exact concentration of the standard.** This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

IV. SAFETY PRECAUTIONS

The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. 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.

V. MATERIALS REQUIRED BUT NOT PROVIDED

1. **Antibody-MMAE Conjugated Stock (Cat No.30712)**
2. Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, etc.
3. Disposable pipette tips suitable for above volume dispensing.
4. Aluminum foil.
5. Deionized or distilled water.
6. Plastic microtiter well cover or polyethylene film.
7. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
8. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

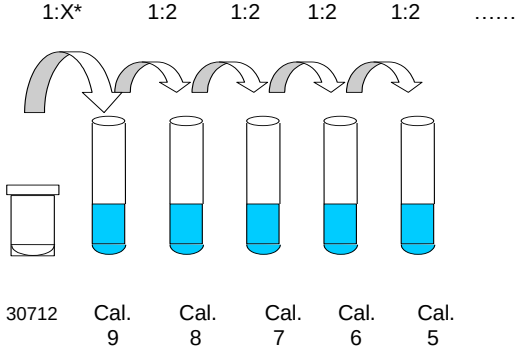
VI. ASSAY PREPARATION

1. 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. Please see REAGENTS section for details.

(3) Using EDI Calibrators:
Reconstitute calibration stock 30712 with **0.5 mL** DI-water. Dilute the reconstituted calibration stock (30712) 1:X* using the zero calibrator (30711) to obtain a level nine calibrator at 4 µg/mL. Further create calibrator level eight to two by 1:2 serial dilutions to obtain these calibrators with concentrations of 2 µg/mL, 1 µg/mL, 0.5µg/mL, 0.25 µg/mL, 0.125 µg/mL, 0.063 µg/mL and 0.032 µg/mL. Assay calibrators should be used within 2 hours and should be stored below -20°C. Do not exceed 3 freeze-thaw cycles.



X* = the concentration of 30712 / 4

(4) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	STD 1	STD 5	STD 9	SAMPLE 4
B	STD 1	STD 5	STD 9	SAMPLE 4
C	STD 2	STD 6	SAMPLE 1	SAMPLE 5
D	STD 2	STD 6	SAMPLE 1	SAMPLE 5
E	STD 3	STD 7	SAMPLE 2	
F	STD 3	STD 7	SAMPLE 2	
G	STD 4	STD 8	SAMPLE 3	
H	STD 4	STD 8	SAMPLE 3	

The validation data of this test was generated by using EDI Antibody-MMAE Conjugated Stock (Cat. No. 30712)! To order this calibrator stock, please order Ab-MMAE Conjugated Stock (Cat. No.30712).

(5) Place a sufficient number of Anti-MMAE antibody coated microwell strips in a holder to determine calibrators and unknown samples in duplicates.

VII. ASSAY PROCEDURE

(1) Add **100 µL** of calibrators and test samples into the designated microwells.

(2) 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.

(3) **Immediately** add **25 µL** of HRP Conjugated MMAE (cat# 30719) to each well. (*Note: no wash step before add the HRP conjugated MMAE*)

(4) 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 **2 hr. ± 10 minutes** at 400 to 450 rpm.

(5) 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.

(6) Add 1**00 µL** of ELISA HRP Substrate into each of the wells.

(7) Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate plate static, at room temperature for **20 minutes**.

(8) Immediately add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.

(9) Read the absorbance at 450 nm.

VIII. PROCEDURAL NOTES

1. It is recommended that all calibrators 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 150 to 200 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.

IX. INTERPRETATION OF RESULTS

It is recommended to use a 4-parameter or log-logit calibration curve fitting.

1. Calculate the average absorbance for each pair of duplicate test results.

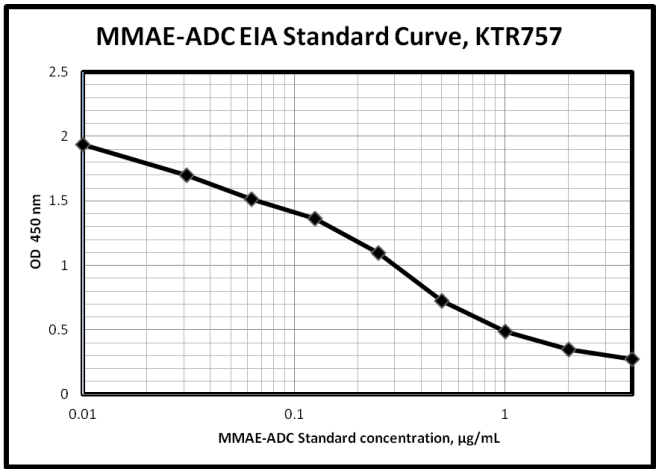
2. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration. Appropriate computer assisted data reduction programs should be used for the calculation of results.

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

X. EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting calibration curve from this MMAE ADC EIA are represented. **This curve should not be used in lieu of calibration curve generated with each assay.**

Well I.D.	OD 450 nm Absorbance		B/B_0
	Readings	Average	
Cal-1: 0.000 µg/mL	2.010 1.866	1.938	100.0%
Cal-2: 0.032 µg/mL	1.729 1.674	1.701	87.8%
Cal-3: 0.063 µg/mL	1.488 1.545	1.516	78.2%
Cal-4: 0.125 µg/mL	1.427 1.294	1.360	70.2%
Cal-5: 0.250 µg/mL	1.039 1.150	1.095	56.5%
Cal-6: 0.500 µg/mL	0.743 0.706	0.724	37.4%
Cal-7: 1.000 µg/mL	0.452 0.521	0.486	25.1%
Cal-8: 2.000 µg/mL	0.346 0.355	0.351	18.1%
Cal-9: 4.000 µg/mL	0.284 0.269	0.276	14.2%



XI. LIMITATION OF THE PROCEDURE

1. This assay requires serum or plasma sample for testing.
2. Serum or plasma samples from different species may show different matrix background. A modification of test procedure may be necessary for measuring rodent samples. Please contact Epitope Diagnostics for technical support.
3. For sample values greater than 4 µg/mL, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100 with zero calibrator matrix. This calibrator zero is available from kit manufacturer. Using a different buffer matrix for sample dilution may cause false high or low value because of matrix effect.). The best assay precision and most reliable test result is located between 15% B/B_0 to 90% B/B_0 of the standard curve.
4. Cell culture or tissue culture samples should be validated with total binding and other performance specifications before being used.
5. The kit calibrators are based on MMAE conjugated antibody or ADC concentration. It is not based on free MMAE concentration. The MMAE-ADC in different linker and DAR may give different curve shift

XII. QUALITY CONTROL

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

XIII. PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of this MMAE ADC EIA as determined by the 2 times standard deviation below the mean of B_0 on 8 duplicate determinations of zero standard (B_0) is approximately 0.0435 µg/mL.

Specificity

This MMAE-ADC EIA doesn't show any cross reactivity to MMAF-ADC, DM1-ADC and DUO-6-ADC.

High Dose "hook" effect

This assay has showed that it didn't have any high dose "hook" effect for MMAE ADC levels up to 1,000 µg/mL.

Precision

The intra-assay precision was validated by measuring three calibrators (L3, L6 and L8) in eight replicate determinations. The CV% is 7.1%, 6.7% and 11.0%.

Linearity

Two samples were diluted with zero calibrator matrix and tested. The results of MMAE ADC dilution recovery value are as follows:

DILUTION	OBSERVED VALUE (µg/mL)	EXPECTED VALUE (µg/mL)	RECOVERY
Calibrator 5	0.250	0.250	-
20% + 80% buffer	0.058	0.050	116.0%
40% + 60% buffer	0.118	0.100	118.0%
60% + 40% buffer	0.141	0.150	94.0%
80% + 20% buffer	0.226	0.200	113.0%
Calibrator 7	1.000	1.000	-
20% + 80% buffer	0.162	0.200	81.0%
40% + 60% buffer	0.379	0.400	94.8%
60% + 40%	0.554	0.600	92.3%

buffer			
80% + 20% buffer	0.893	0.800	111.6%

Spike Recovery
Calibrator level 4 and 6 is equal volume mixed with calibrator level 3, 5, and 7 and tested. The results are as follows:

Spiked Sample	OBSERVED VALUE (µg/mL)	EXPECTED VALUE (µg/mL)	RECOVERY
Cal. 4	0.125	0.125	-
Cal. 4+Cal.-3 (0.063)	0.089	0.094	94.7%
Cal. 4+Cal.-5 (0.250)	0.183	0.188	97.6%
Cal. 4+Cal.-7 (1.000)	0.460	0.563	81.7%
Cal. 6	0.500	0.500	-
Cal. 6+Cal.-3 (0.063)	0.268	0.282	95.0%
Cal. 6+Cal.-5 (0.250)	0.345	0.375	92.0%
Cal. 6+Cal.-7 (1.000)	0.647	0.750	86.3%

XIV. WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

XV. REFERENCES

1. Sandhya Girish, et al. Clinical pharmacology of trastuzumab emtansine (T-DM1): an antibody–drug conjugate in development for the treatment of HER2-positive cancer. Cancer Chemother Pharmacol (2012) 69:1229–1240

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This product is developed and manufactured by
Epitope Diagnostics, Inc.
San Diego, CA 92121, USA

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
Read instructions before use	Use by
	Lot No.

MMAE- ADC EIA: Condensed Assay Protocol

1. **100 µl calibrators and unknown samples**
   ↓ *Incubate @ RT for 30 min on ELISA plate shaker*
2. **25 µl MMAE Tracer**
   ↓ *Incubate @ RT for 2 hours on ELISA plate shaker Wash 5 x*
3. **100 µl TMB Substrate**
   ↓ *Incubate @ RT for 20 min static*
4. **100 µl Stop Solution** *Immediately*
   ↓
5. **Read absorbance at 450 nm** *within 10 minutes*