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EDI™ Trastuzumab Emtansine EIA Kit

Enzyme Immunoassay (EIA) for the Quantitative Measurement of Trastuzumab Emtansine Level in Human Serum

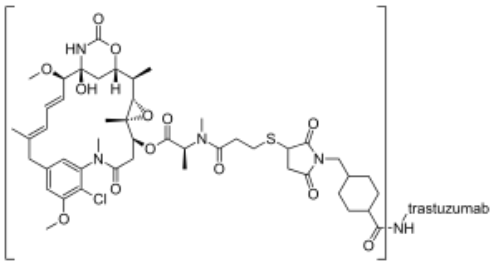
REF **KT-706** EU: IVD CE Σ 96 +2 +8 °C

I. INTENDED USE

This test kit is intended for use in the quantitative determination of Trastuzumab emtansine in human serum sample. It is useful for clinical monitoring the therapeutic drug concentration for a precision medical treatment.

II. ASSAY PRINCIPLE

This EIA kit is designed, developed and produced for the quantitative measurement of cancer treatment drug, Trastuzumab emtansine or other antibody DM1 conjugate in serum. The assay utilizes the competitive immunoassay technique with an antibody that exclusively binds to DM1.



Assay calibrators (antibody DM1 conjugate) and test serum samples are added directly to wells of a microtiter plate that is coated with specific anti-DM1 antibody. Subsequently, a horseradish peroxidase (HRP) conjugated DM1 is added to each well. During the incubation period, the antibody DM1 conjugate competes with the HRP conjugated DM1 for the limited binding sites of anti-DM1 antibody. An immune complex of well coated "anti-DM1 antibody – HRP conjugated DM1" 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-DM1 conjugate in the test sample. A calibration curve is generated by plotting the absorbance versus the respective antibody-DM1 conjugate concentration for each calibrator on a 4-parameter or log-logit curve fitting. The concentration of antibody-DM1 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-DM1 Antibody Coated Microplate (Cat. No. 30750)
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. HRP Conjugated DM1 (Cat. No. 30683)
One vial containing **3 mL** of ready to use HRP labeled DM1 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-DM1 Conjugated Calibrator Zero (Cat. No.30701b)
One bottle containing **120 mL** calibrator zero (30701b). This reagent is used for diluting the calibration stock to 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-DM1 Conjugated Calibrator Stock (Cat. No. 30702)
One vial (30702) containing the calibration stock of antibody-DM1-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. 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 (semi-automatic) washing system.
7. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

VI. SPECIMEN COLLECTION

Only 10 µL of human serum is required for measurement. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples may be stored at –20ºC or below until measurement. Avoid more than three freeze-thaw cycles of specimen.

VII. ASSAY PREPARATION

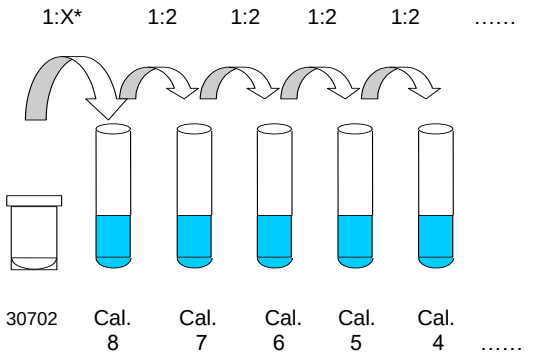
1. Reagent Preparation

(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 30702 with **0.5 mL** DI-water. Dilute the reconstituted calibration stock (30702) 1:X* using the zero calibrator (30701) to obtain a level-8 standard at 1 µg/mL. Further create calibrator level seven to two by 1:2 serial dilutions to obtain these calibrators with concentrations of 0.5 µg/mL, 0.25 µg/mL, 0.125 µg/mL, 0.063 µg/mL, 0.031 µg/mL, 0.016 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 30702 / 1

(4) Test Configuration

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

(5) Place a sufficient number of Anti-DM1 antibody coated microwell strips in a holder to determine calibrators and unknown samples in duplicates.

2. Patient Sample Preparation

Because of high sensitivity of this assay, patient serum sample is recommended to be diluted at 1:50 and/or 1:100 before test. The dilution matrix should be the calibrator zero matrix.

VIII. ASSAY PROCEDURE

(1) Add **100 µL** of calibrators and the diluted 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 DM1 (cat# 30683) to each well. (*Note: no wash step before add the HRP Conjugated DM1*)

(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.

IX. 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.

X. INTERPRETATION OF RESULTS

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

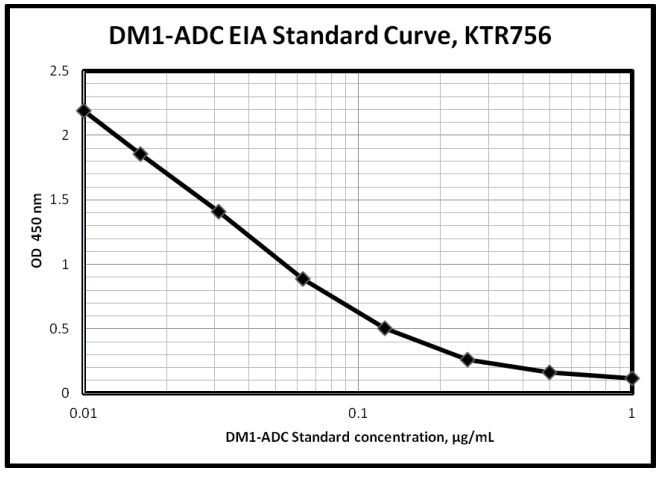
1. Calculate the average absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the corrected absorbance of all calibration levels on the ordinate against the calibrator concentration. Appropriate computer assisted data reduction programs should be used for the calculation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

The Trastuzumab emtansine or antibody-DM1 conjugate concentrations for the test samples are read directly from the calibration curve using their respective corrected absorbance.

XI. EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting calibration curve from this DM1 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	2.234 2.150	2.192	100.0%
Cal-2: 0.016 µg/mL	1.753 1.960	1.857	84.7%
Cal-3: 0.032 µg/mL	1.446 1.375	1.410	64.3%
Cal-4: 0.063 µg/mL	0.902 0.868	0.885	40.4%
Cal-5: 0.125 µg/mL	0.495 0.520	0.508	23.2%
Cal-6: 0.250 µg/mL	0.260 0.270	0.265	12.1%
Cal-7: 0.500 µg/mL	0.157 0.165	0.161	7.3%
Cal-8: 1.000 µg/mL	0.111 0.119	0.115	5.2%



XII. 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 0.5 µg/mL, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100 with calibrator zero. 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 10% B/B_0 to 85% B/B_0 of the standard curve.
4. The kit calibrators 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.
5. Trastuzumab emtansine dosage must be established for desired patient concentration. Proper dosage is ultimately left to the discretion of the doctor.

XIII. QUALITY CONTROL

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

XIV. PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of this DM1 ADC EIA as determined by the 2 times calibrator deviation below the mean of B_0 on 8 duplicate determinations of zero calibrator (B_0) is approximately 0.024 µg/mL.

The measurement rage for this DM1 ADC EIA is 0.024 µg/mL to 1.000 µg/mL

Specificity

This assay measures the intact Trastuzumab emtansine. It does not detect free DM1 and the monoclonal antibody. This DM1-ADC EIA doesn't show any cross reactivity to MMAE-ADC, MMAF-ADC, DUO-3 ADC, and DUO-6 ADC.

High Dose "hook" effect

This assay has showed that it didn't have any high dose "hook" effect for DM1 ADC levels up to 1,000 µg/mL.

Precision

The intra-assay precision was validated by measuring three calibrators (L3, L5 and L7) in six replicate determinations. The CV% is 6.8%, 6.2% and 8.9%.

Linearity

Two samples were diluted with calibrator zero and tested. The results of DM1 ADC dilution recovery value are as follows:

DILUTION	OBSERVED VALUE (µg/mL)	EXPECTED VALUE (µg/mL)	% RECOVERY
Calibrator 6	0.25	-	-
20% + 80% buffer	0.045	0.05	111.1%
40% + 60% buffer	0.104	0.1	96.2%
60% + 40% buffer	0.149	0.15	100.7%

80% + 20% buffer	0.219	0.2	91.3%
Calibrator 8	1	-	-
20% + 80% buffer	0.225	0.2	88.9%
40% + 60% buffer	0.488	0.4	82.0%
60% + 40% buffer	0.578	0.6	103.8%
80% + 20% buffer	0.735	0.8	108.8%

Spike Recovery
Calibrator level 5 and 7 is equal volume mixed with standard level 4, 6, 8 and tested. The results are as follows:

Spiked Sample	OBSERVED VALUE (µg/mL)	EXPECTED VALUE (µg/mL)	RECOVERY
Calibrator 5	0.125	0.125	-
Cal. 5+Cal.-4 (0.063)	0.085	0.094	90.4%
Cal. 5+Cal.-6 (0.250)	0.193	0.188	102.9%
Cal. 5+Cal.-8 (1.000)	0.637	0.563	113.2%
Calibrator 7	0.500	0.500	-
Cal. 5+Cal.-4 (0.063)	0.328	0.282	116.5%
Cal. 5+Cal.-6 (0.250)	78	0.375	100.8%
Cal. 5+Cal.-8 (1.000)	0.719	0.750	95.9%

XV. 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.

XVI. 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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CE EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Manufacturer	No. of tests
REF Catalog Number	Keep away from heat and direct sun light
CONC Concentrate	Store at
IVD In Vitro Diagnostic Device	Use by
Read instructions before use	LOT Lot No.
EC REP Authorized Representative In Europe	

DM1- ADC EIA: Condensed Assay Protocol

1. **100 µl calibrators and unknown samples**
   ↓ *Incubate @ RT for 30 min on ELISA plate shaker*
2. **25 µl Tracer Antibody**
   ↓ *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*