

KRIBIOLISA™ Trastuzumab Emtansine ELISA

REF KBI1017-03

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of
Trastuzumab Emtansine in human serum and plasma.

RUO	For Research Use only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBI1017-3



96 tests



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Introduction:

Trastuzumab emtansine, formerly called Trastuzumab-DM1 (T-DM1) is a first-in-class HER2 antibody drug conjugate (ADC) comprised of Genentech's Trastuzumab antibody linked to ImmunoGen's cell-killing agent, DM1. T-DM1 combines two strategies-- anti-HER2 activity and targeted intracellular delivery of the potent antimicrotubule agent, DM1 (a maytansine derivative)--to produce cell cycle arrest and apoptosis. Trastuzumab emtansine is marketed under the brand name Kadcyla and is indicated for use in HER2-positive, metastatic breast cancer patients who have already used taxane and/or Trastuzumab Emtansine for metastatic disease or had their cancer recur within 6 months of adjuvant treatment.

Intended Use:

The KRIBIOLISA™ Trastuzumab Emtansine ELISA is used as an analytical tool for quantitative determination of Trastuzumab Emtansine in human serum and plasma.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Recombinant HER2-ECD are pre-coated onto microwells. Samples and standards are pipetted into microwells and human Trastuzumab Emtansine present in the sample are bound by the capture protein. Then, a HRP (horseradish peroxidase) conjugated to DM1 is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Trastuzumab Emtansine in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Recombinant HER2 protein Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Recombinant HER2 protein.	1 x 96 wells
T-DM1 Standard	T-DM1 in a buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodiroxane – lyophilized (100 ug/ml)	2 vials
Anti-DM1:HRP Conjugate	Anti-DM1 conjugated to Horseradish Peroxidase with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodiroxane.	12 ml
Standard Diluent	Buffered protein base with 1:100 dilution human serum and protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodiroxane	10 ml
Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodiroxane	50 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized chromogen	12 ml
Stop Solution	0.73M Phosphoric acid	12 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2°C to 8°C for stability.

2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.



Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

For Cell Culture Supernatant – If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation – Serum and Plasma samples have to be diluted 1:100 (v/v), e.g. for 1:100 (1 ul sample + 99 ul sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.
4. **Standards Preparation:** Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent to obtain a concentration of 1 ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 320 ul of original **Standard (100 ug/ml)** with 180 ul of Standard Diluent to generate a **100 ug/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
100 ug/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit + 1ml of Standard Diluent
50 ug/ml	Standard No.9	320 ul Reconstituted Standard (100 ug/ml) + 180 ul Standard Diluent
25 ug/ml	Standard No.8	250 ul Standard No.9 + 250 ul Standard Diluent
12.5 ug/ml	Standard No.7	250 ul Standard No.8 + 250 ul Standard Diluent
6.25 ug/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard Diluent
3.125 ug/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent
1.56 ug/ml	Standard No.4	250 ul Standard No.5 + 250 ul Standard Diluent
0.78 ug/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard Diluent
0.39 ug/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard Diluent
0.195 ug/ml	Standard No.1	250 ul Standard No.2 + 250 ul Standard Diluent
0 ug/ml	Standard No.0	250 ul Standard No.1 + 250 ul Standard Diluent

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Trastuzumab Emtansine. High Dose Hook Effect is due to excess of antibody for very high concentrations of Trastuzumab Emtansine present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Trastuzumab Emtansine concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Trastuzumab Emtansine.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C
2. Add **100 ul** of **Standards or Samples** into the respective wells.
3. Cover the plate and incubate for 60 minutes at 37°C
4. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
5. Add **100 ul** of **Anti-DM1:HRP Conjugate** into each well.
6. Cover the plate and incubate for 60 minutes at 37°C
7. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
8. Add **100 ul** of **TMB Substrate** in each well.
9. Incubate the plate at 37°C for 30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate reader.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Trastuzumab Emtansine concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Trastuzumab Emtansine Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 640 ug/ml standard.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Sensitivity:

Limit Of Quantification: It is defined as the lowest concentration of an analyte that can be determined with an acceptable repeatability and the LOQ was found to be less than 0.156 ug/ml

Linearity:

Standards provided in the kit will be used for measuring the linearity range of Trastuzumab Emtansine present in matrix.

Precision:

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (10 ug/ml), medium (80 ug/ml) and high (640 ug/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<12%	<10%
Medium	<10%	<5%
High	<10%	<5%

Safety Precautions:

- **This kit is for research use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1% w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.



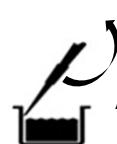
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.



3. Pipette 100 ul Standards / Samples into the respective wells.



4. Cover plate and incubate for 60 min at 37°C.



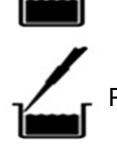
5. Aspirate and wash wells 4 times with Wash Buffer (1X).



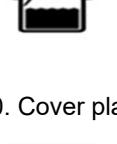
6. Pipette 100 ul Anti-DM1:HRP into each well.



7. Cover plate and incubate for 60 min at 37°C.



8. Aspirate and wash wells 4 times with Wash Buffer (1X).



9. Pipette 100 ul TMB Substrate into each well.



10. Cover plate and incubate for 30 min at 37°C.



11. Pipette 100 ul Stop Solution into each well.



12. Read absorbance at 450nm with a microplate reader within 30 min of stopping reaction.



Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ug/ml Trastuzumab Emtansine equivalent
1A	zero std			
2A	zero std			
1B	100 ug/ml			
2B	100 ug/ml			
1C	50 ug/ml			
2C	50 ug/ml			
1D	25 ug/ml			
2D	25 ug/ml			
1E	12.5 ug/ml			
2E	12.5 ug/ml			
1F	6.25 ug/ml			
2F	6.25 ug/ml			
1G	3.125 ug/ml			
2G	3.125 ug/ml			
1H	1.56 ug/ml			
2H	1.56 ug/ml			
3A	Sample			
4A	Sample			
3B	Sample			
4B	Sample			

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