

Human Therapeutic IgG Mertansine ADC ELISA Kit

Item No. 502872

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
401151	Mertansine-Human IgG Standard	1 vial/150 ng	-20°C
401149	Anti-Mertansine Biotin Conjugate	1 vial	-20°C
401175	Anti-Human IgG Precoated 96-Well Strip Plate	1 plate	4°C
400664	Streptavidin Poly-HRP	1 vial/1.5 ml	4°C
400108	Immunoassay Buffer D Concentrate (5X)	3 vials/10 ml	4°C
400035	Polysorbate 20	1 vial/3 ml	RT
400074	TMB Substrate Solution	1 vial/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Human Therapeutic IgG Mertansine ADC ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.* safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 12)

INTRODUCTION

Background

Mertansine is a semisynthetic maytansinoid antimitotic agent.[1,2] It disrupts mitotic spindle formation and induces cell cycle arrest and cell death in cancer cells *in vitro*. Mertansine has commonly been used as a payload in antibody-drug conjugates (ADCs), which have been used to selectively target and destroy cancer cells *in vitro* and *in vivo*.[3-5] In ADCs, the payload is modified to create an antibody-linker-payload complex in which the antibody directs the complex to target cells, while the payload stays inactive until cleaved from the linker intracellularly.[6] Mertansine conjugated to therapeutic antibodies has shown potent anti-tumor activity in several cancers.[5,6] For example, mertansine linked to the HER2 antibody trastuzumab (T-DM1) reduces the proliferation of HER2-overexpressing cancer cells *in vitro* and reduces tumor growth in mouse xenograft models of HER2-overexpressing cancers.[7,8] Formulations containing mertansine-based ADCs have been used in the treatment of $HER2^+$ metastatic breast cancer.

About This Assay

Cayman's Human Therapeutic IgG Mertansine ADC ELISA Kit is an immunometric (*i.e.* sandwich) assay that can be used for the quantification of mertansine-human IgG complexes (mertansine ADCs) in mouse, rat, rabbit, and non-human primate serum and cell culture supernatant. The standard curve spans the range of 0.47-30 ng/ml, with a lower limit of detection (LLOD) of 0.2 ng/ml.

Please note that the standard included in this kit consists of mertansine conjugated to a recombinant humanized IgG1 monoclonal antibody and is suitable for semi-quantitative analysis. For fully quantitative results, users should generate a standard curve using their own sample-specific mertansine-conjugated antibody, ensuring the dose range matches the kit's provided standard.

Principle Of This Assay

This immunometric assay is based on a double-antibody “sandwich” technique. Each well of the microwell plate supplied with the kit has been coated with a polyclonal antibody specific for human IgG. This antibody will bind any mertansine-antibody conjugates introduced into the well regardless of antibody subtype and or linker. A second polyclonal antibody conjugated to biotin, which recognizes mertansine, is added to the well forming a “sandwich”. The “sandwich” is immobilized on the plate, and the excess reagents are washed away. Streptavidin conjugated to horseradish peroxide (HRP) is then added to the wells. The streptavidin binds to the biotinylated “sandwich,” and the amount of bound streptavidin HRP is determined using the chromogenic HRP substrate 3,3’,5,5’-tetramethylbenzidine (TMB). After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color is directly proportional to the amount of bound antibody-biotin conjugate, which is proportional to the concentration of mertansine ADCs.

$$\text{Absorbance} \propto [\text{streptavidin HRP}] \propto [\text{anti-mertansine biotin}] \propto [\text{mertansine ADC}]$$

A schematic of this process is shown in Figure 1, on page 9.

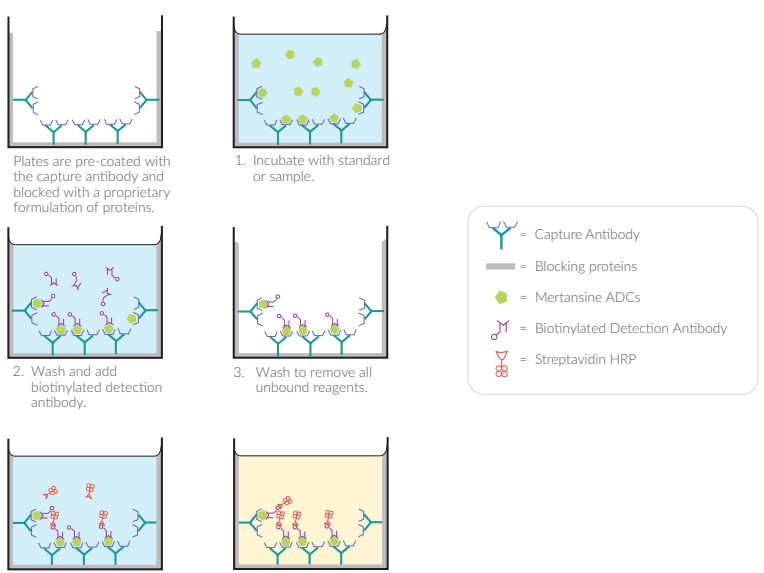


Figure 1. Schematic of the ELISA

Definition of Key Terms

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of analyte.

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration corresponding to two standard deviations higher than the mean zero absorbance value.

Lower Limit of Quantification (LLOQ): the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the mean zero absorbance (450 nm) + (1.64 x S.D.).

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for at least two months. *NOTE: It is normal for the concentrated buffer to contain crystalline salts. These will completely dissolve upon dilution with ultrapure water. Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

1. **Assay Buffer**

   Dilute the contents of each vial of Immunoassay Buffer D Concentrate (5X) (Item No. 400108) with 40 ml of ultrapure water and add 50 µl of Polysorbate 20 (Item No. 400035). Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X)**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20.

Sample Preparation

This assay has been validated in rabbit, rat, monkey, pig, and mouse sera, mouse serum, cell culture supernatants, and cell culture media. Serum and plasma samples must be diluted at least 1:10 in Assay Buffer prior to use in the assay.

For other samples, it is recommended to determine a minimum sample dilution before embarking on a large number of measurements. To determine the minimum sample dilution, dilute one or two test samples to obtain several different dilutions for each sample. The dilution factor where the change in the final calculated mertansine ADC concentration is consistent, differing by 20% or less than the previous dilution, is the minimum required dilution for that sample type. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C .

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Mertansine-Human IgG Standard

The included mertansine-human IgG standard is intended for semi-quantitative use. For quantitative measurements, create a standard curve using your own conjugate at a similar dose range to the provided standard.

Reconstitute the lyophilized Mertansine-Human IgG Standard (Item No. 401151) with 1.0 ml of Assay Buffer and mix gently. The concentration of this solution (the bulk standard) will be 150 ng/ml. The reconstituted standard will be stable for at least two weeks when stored at 4°C.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 800 µl Assay Buffer to tube #1 and 500 µl Assay Buffer to tubes #2-8. Transfer 200 µl of the bulk standard to tube #1 and mix gently. Serially dilute the standard by removing 500 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-7. Do not add any standard to tube #8. This tube is the background control. These diluted standards should not be stored for more than two hours.



Figure 2. Preparation of the mertansine-human IgG standards

Anti-Mertansine Biotin Conjugate

Reconstitute the lyophilized Anti-Mertansine Biotin Conjugate (Item No. 401149) with 12 ml of Assay Buffer and mix gently. The conjugate solution will be stable for at least two weeks when stored at 4°C.

Streptavidin Poly-HRP

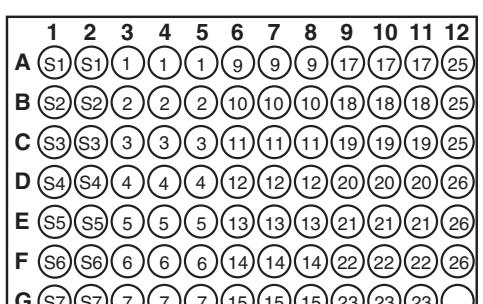
Streptavadin Poly-HRP (Item 400664) is supplied as a 20X solution. On the day of the assay, bring the vial to room temperature. Immediately prior to use dilute 600 µl of this solution with 11.4 ml of Assay Buffer to make a 1X streptavidin poly-HRP working solution. Store unused Strepavidin Poly-HRP (20X) at 4°C. It will be stable for at least two weeks.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight-point standard curve run in duplicate. Each sample should be assayed at a minimum of two dilutions, and each dilution should be assayed at least in duplicate. For statistical purposes, assaying the samples in triplicate is recommended.

A suggested plate format is shown in Figure 3, on page 17. The user may vary the location and type of wells present as necessary for each particular experiment. It is suggested that the contents of each well be recorded on the template sheet provided (see page 26).



S1-S8 = Standard Wells
1-26 = Sample Wells

Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Standards and Samples and First Incubation

1. Pipette 100 µl of the standards or samples into the appropriate wells on the plate.
2. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for two hours at room temperature on an orbital shaker.

Addition of the Biotin Conjugate and Second Incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of the reconstituted biotin conjugate to each well of the plate.
3. Cover the plate with the 96-Well Cover Sheet and incubate for one hour at room temperature on an orbital shaker.

Addition of the Streptavidin Poly-HRP and Third Incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Prepare a 1X working solution of Streptavidin Poly-HRP as described on page 15.
3. Add 100 µl of the Streptavidin Poly-HRP (1X) working solution to each well of the plate.
4. Cover the plate with the 96-Well Cover Sheet and incubate for 30 minutes at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes, protected from light.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively, a spreadsheet program can be used.

Calculations

Plot the Standard Curve and Determine the Sample Concentration

Average the absorbance readings from the standards and samples. Substract the average background absorbance (standard 8, 0 ng/ml) from the average absorbance of all standards and samples. Using computer reduction software, plot background-subtracted absorbance (linear y-axis) *versus* concentration (log x-axis) for standards (S1-S7) and fit the data with a four-parameter logistic (4PL) curve fit. Using the equation of the curve, calculate the concentration of mertansine ADCs in each sample, making sure to correct for any sample dilution.

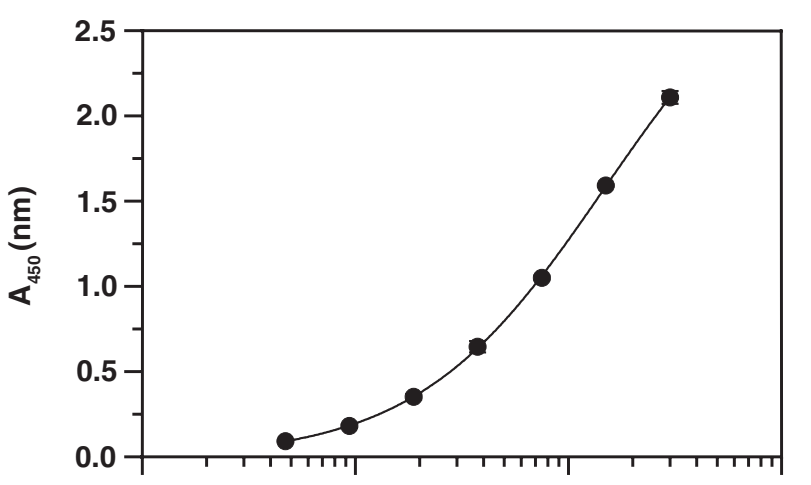
Performance Characteristics

Representative Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve with each experiment. Do not use the data below to determine the values of your samples.

Mertansine-Human IgG Standards (ng/ml)	Absorbance (450 nm)	Background-corrected Absorbance	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
30	2.221	2.109	8.1	7.2
15	1.705	1.593	5.6	7.0
7.5	1.164	1.052	3.8	5.3
3.75	0.759	0.647	4.1	6.0
1.88	0.465	0.353	2.9	8.4
0.94	0.294	0.182	3.5	7.1
0.47	0.206	0.094	3.7	7.5
0	0.112	0		

Table 1. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve



Assay Range = 0.47-30 ng/ml
Sensitivity (defined as LLOQ) = 0.47 ng/ml
Lower Limit of Detection (LLOD) = 0.2 ng/ml

Figure 4. Typical standard curve

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of replicates	A. Trace organic contaminants in the water B. Poor pipetting/technique
High background wells (>0.30 O.D.)	A. Poor washing; ensure proper washing B. Exposure of background wells to standards or samples C. Polysorbate 20 was not added to the Assay Buffer
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water B. Dilution error in preparing reagents C. Streptavidin Poly-HRP was not freshly diluted prior to use
Poor development (low signal) of samples	A. Samples do not contain mertansine-human IgG conjugate B. The dilution of samples is too high
Analyses of two dilutions of a biological sample do not agree (*i.e.* more than 20% difference)	Interfering substances are present; determine minimal dilution for that sample type

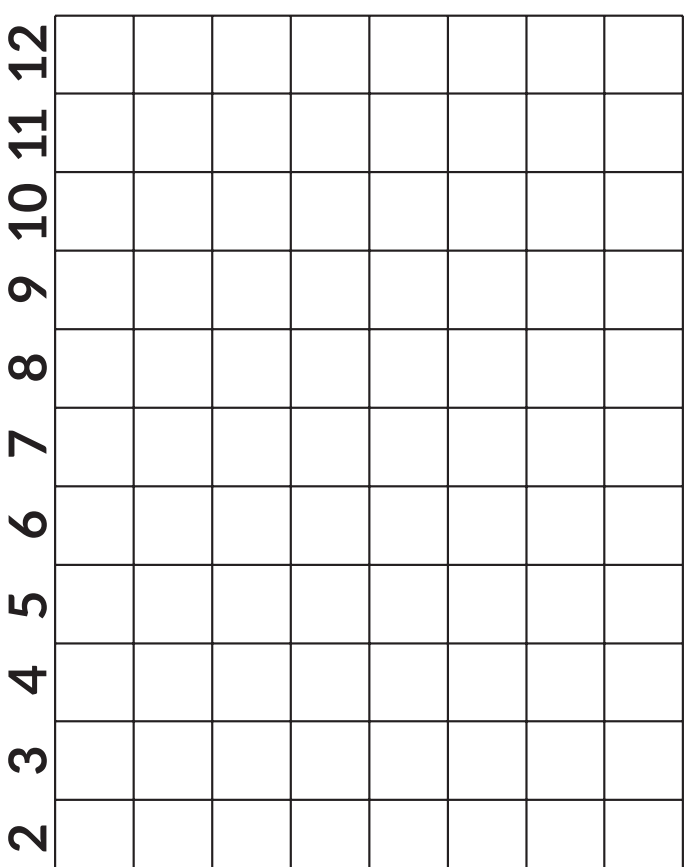
References

1. Widdison, W.C., Wilhelm, S.D., Cavanagh, E.E., *et al.* Semisynthetic maytansine analogues for the targeted treatment of cancer. *J. Med. Chem.* **49(14)**, 4392-4408 (2006).
2. Lopus, M. Antibody-DM1 conjugates as cancer therapeutics. *Cancer Lett.* **307(2)**, 113-115 (2011).
3. Berdeja, J.G. Lorvotuzumab mertansine: Antibody-drug-conjugate for CD56+ multiple myeloma. *Front. Biosci.* **19(1)**, 163-170 (2014).
4. Mckertish, C.M. and Kayser, V. A novel dual-payload ADC for the treatment of HER2+ breast and colon cancer. *Pharmaceutics* **15(18)**, 2020 (2023).
5. Huhe, M., Lou, J., Zhu, Y., *et al.* A novel antibody-drug conjugate, HcHAb18-DM1, has potent anti-tumor activity against human non-small cell lung cancer. *Biochem. Biophys. Res. Commun.* **513(4)**, 1083-1091 (2019).
6. Sassoon, I. and Blanc, V. Antibody-drug conjugate (ADC) clinical pipeline: A review. *Methods Mol. Biol.* **1045**, 1-27 (2013).
7. Li, G., Guo, J., Shen, B.-Q., *et al.* Mechanisms of acquired resistance to trastuzumab emtansine in breast cancer cells. *Mol. Cancer Ther.* **17(7)**, 1441-14453 (2018).
8. Lewis Phillips, G.D., Li, G., Dugger, D.L., *et al.* Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res.* **68(22)**, 9280-9290 (2008).

Assay Summary

Procedure	Standards and Samples
Mix all reagents gently	--
Add standards/samples to plate	100 µl
Incubate	Seal the plate and incubate for 2 hours at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add reconstituted Anti-Mertansine Biotin Conjugate	100 µl
Incubate	Seal the plate and incubate for 1 hour at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add Streptavidin Poly-HRP (1X)	100 µl
Incubate	Seal the plate and incubate for 30 minutes at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add TMB Substrate Solution	100 µl
Develop	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker, protected from light
DO NOT WASH. Add HRP Stop Solution	100 µl
Read	Read absorbance at 450 nm

Table 2. Assay summary



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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