Version 25-02/25

User's Manual

MMAF ADC EIA Kit

REF DEIABL313

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.

Creative Diagnostics
Address: 45-1 Ramsey Road, Shirley, NY 11967, USA
Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) Fax: 1-631-938-8221
Email: info@creative-diagnostics.com Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

This test kit is intended for use in the quantitative determination of antibody-MMAF-conjugate level in test sample. It is useful for preclinical and clinical pharmacology study of MMAF 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 MMAF-ADC and mouse monoclonal antibody based MMAF-ADC can be measured with this kit.

Principles of Testing

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

Assay calibrators (antibody MMAF conjugate) and test serum samples are added directly to wells of a microtiter plate that is coated with specific anti-MMAF antibody. Subsequently, a horseradish peroxidase (HRP) conjugated MMAF is added to each well. During the incubation period, the antibody MMAF conjugate competes with the HRP conjugated MMAF for the limited binding sites of anti-MMAF antibody. An immune complex of well coated "anti-MMAF antibody - HRP conjugated MMAF" 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-MMAF conjugate in the test sample. A calibration curve is generated by plotting the absorbance versus the respective antibody-MMAF conjugate concentration for each calibrator on a 4-parameter or loglogit curve fitting. The concentration of antibody-MMAF conjugate in test samples is determined directly from this calibration curve.

Reagents And Materials Provided

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-MMAF Antibody Coated Microplate

One microplate with twelve by eight strips (96 wells total) coated with specific anti-MMAF 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 MMAF

One vial containing 3 mL of ready to use HRP labeled MMAF 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

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

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

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-MMAF Conjugated Zero Calibrator (Cat. No. CD41832)

One vial containing 30mL zero calibrator (CD41832). 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 2-8°C and is stable until the expiration date on the kit box.

7. Antibody-MMAF Conjugated Calibration Stock (Cat. No. CD41833) - Not provided in the kit (optional)

One vial (CD41833) containing the calibration stock of antibodyMMAF-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.

Materials Required But Not Supplied

1. Antibody-MMAF Conjugated Stock.
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 (semiautomatic) washing system.
8. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

Storage

Unopened kit: Store at 2-8°C. Do not use the kit beyond the expiration date.

Reagent Preparation

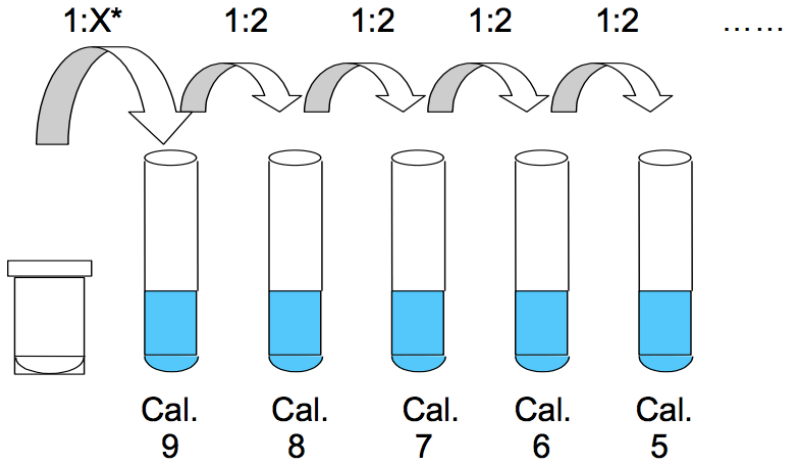
(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.

(3) Using Creative Diagnostics Calibrators:

Reconstitute calibration stock CD41833 with 0.5 mL DI-water. Dilute the reconstituted calibration stock (CD41833) 1:X* using the zero calibrator (CD41832) to obtain a level nine calibrator at 2 µg/mL. Further create calibrator level eight to two by 1:2 serial dilutions to obtain these calibrators with concentrations of 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL, 0.125 µg/mL, 0.063 µg/mL, 0.032 µg/mL and 0.016 µg/mL.

Assay calibrators should be used within 2 hours and should be stored below -20°C. Do not exceed 3 freezethaw cycles.



X* = the concentration of CD41833 / 2

(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 MMAF Conjugated Stock (Cat. No. CD41833)! To order this calibrator stock, please order Ab-MMAF Conjugated Stock (Cat. No. CD41833).

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

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 MMAF to each well. (Note: no wash step before add the HRP conjugated MMAF)

(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 100 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.

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.

Quality Control

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

Interpretation Of Results

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

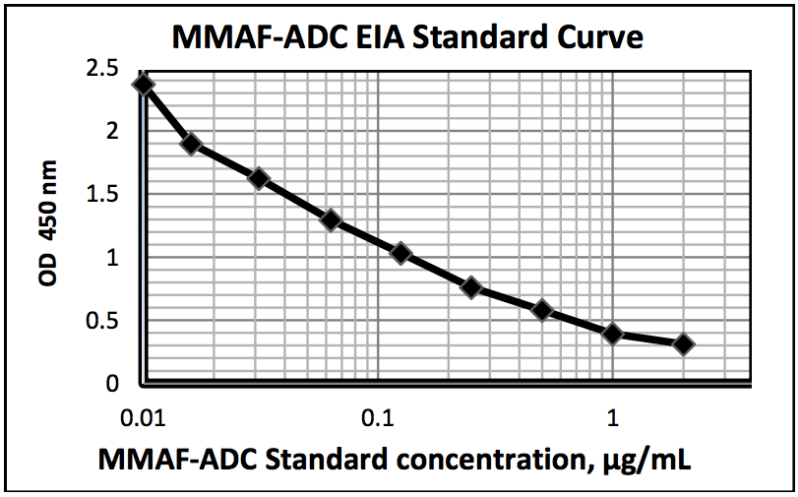
1. Calculate the average absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration. Appropriate computer assisted data reduction programs should be used for the calculation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

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

Typical Standard Curve

A typical absorbance data and the resulting calibration curve from this MMAF 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	2.362 2.373	2.368	100.0%
Cal-2: 0.016 µg/mL	1.899 1.899	1.899	80.2%
Cal-3: 0.032 µg/mL	1.623 1.626	1.624	68.6%
Cal-4: 0.063 µg/mL	1.320 1.265	1.293	54.6%
Cal-5: 0.125 µg/mL	1.030 1.034	1.032	43.5%
Cal-6: 0.250 µg/mL	0.721 0.799	0.760	32.1%
Cal-7: 0.500 µg/mL	0.598 0.561	0.580	24.5%
Cal-8: 1.000 µg/mL	0.413 0.373	0.393	16.6%
Cal-9: 2.000 µg/mL	0.291 0.331	0.311	13.1%



Performance Characteristics

High Dose "hook" effect

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

Precision

The intra-assay precision was validated by measuring three calibrators (L3, L4 and L7) in six replicate determinations. The CV% is 5.5%, 4.9% and 6.1%.

Sensitivity

The analytical sensitivity (LLOD) of this MMAF ADC EIA as determined by the 2 times standard deviation below the mean of B0 on 12 duplicate determinations of zero standard (B0) is approximately 0.019 μg/mL.

Specificity

This MMAF-ADC EIA doesn't show any cross reactivity to MMAEADC, DM1-ADC, DUO-3-ADC, and DUO-6-ADC.

Linearity

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

DILUTION	OBSERVED VALUE (μg/mL)	EXPECTED VALUE (μg/mL)	RECOVERY
Calibrator 5	0.125	0.125	-
20% + 80% buffer	0.024	0.025	96.0%
40% + 60% buffer	0.045	0.050	90.0%
60% + 40% buffer	0.049	0.075	105.3%
80% + 20% buffer	0.101	0.100	101.0%
Calibrator 7	0.500	0.500	-
20% + 80% buffer	0.109	0.100	109.0%
40% + 60% buffer	0.215	0.200	107.5%
60% + 40% buffer	0.347	0.300	111.3%
80% + 20% buffer	0.473	0.400	115.5%

Recovery

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

Spiked Sample	OBSERVED VALUE (µg/mL)	EXPECTED VALUE (µg/mL)	RECOVERY
Cal. 5	0.125	0.125	-
Cal. 5+Cal.-4 (0.063)	0.104	0.094	110.6%
Cal. 5+Cal.-6 (0.250)	0.208	0.188	110.9%
Cal. 5+Cal.-8 (1.000)	0.626	0.563	100.1%
Cal. 7	0.500	0.500	-
Cal. 7+Cal.-4 (0.063)	0.331	0.282	117.6%
Cal. 7+Cal.-6 (0.250)	0.407	0.375	108.5%
Cal. 7+Cal.-8 (1.000)	0.698	0.750	93.1%

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.

Limitations

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 2 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/B0 to 85% B/B0 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 MMAF conjugated antibody or ADC concentration. It is not based on free MMAF concentration. The MMAF-ADC in different linker and DAR may give different curve shift.