

MMAF-ADC Quantitative Detection ELISA Kit

Pack Size: 96 tests

Catalog Number: PKA-A002

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

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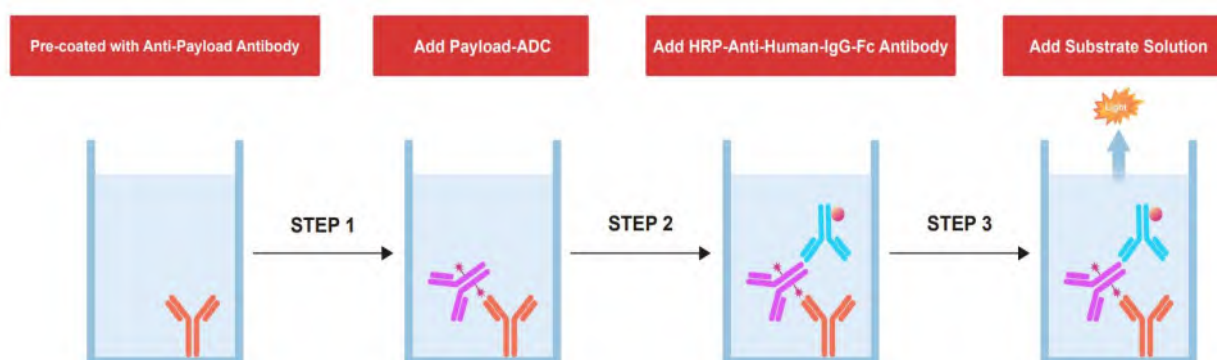
【Intended Use】

The MMAF-ADC Quantitative Detection ELISA Kit is intended for the quantitative detection of intact MMAF-ADC in cynomolgus monkey serum and plasma and serves as an effective tool for intact ADC quantification during ADC drug research and development. It is intended for research use only (RUO).

【Assay Principle】

This kit is used to measure the levels of MMAF-ADC using a Sandwich-ELISA format. The microplate is pre-coated with an Anti-MMAF Antibody, which captures MMAF-ADC present in standards and samples, then an HRP-Anti-Human-IgG-Fc Antibody is added to bind the captured MMAF-ADC, forming an antibody-antigen-biotinylated antibody complex. Following additional washes, a substrate is added for color development. The reaction is stopped with stop solution, and the color changes from blue to yellow. Absorbance is measured at 450 nm with a 630 nm reference. The absorbance signal is directly proportional to the amount of MMAF-ADC in the sample.

Figure 1. ELISA Assay Principle



【Materials Provided】

Table 1. Materials Provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
PKA002-C01	Pre-coated Anti-MMAF Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
PKA002-C02	MMAF-ADC Standard	20 µg	Powder	2-8°C	-70°C
PKA002-C03	HRP-Anti-Human-IgG-Fc Antibody	20 µg	Powder	2-8°C, avoid light	-70°C, avoid light
PKA002-C04	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
PKA002-C05	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
PKA002-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
PKA002-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

【Reagents and Consumables / Equipment Required but not Provided】

Single-or dual-wavelength microplate reader with 450nm and 630nm filters;

Incubator;

Single- or multi-channel micropipettes;

10 µL, 200 µL, and 1000 µL precision pipettes;

Centrifuge tubes;

Timer;

Reagent bottle;

Deionized water or ultrapure water;

【Storage】

1. Store the unopened kit at 2-8°C upon receipt.
2. Locate the expiration date on the outer packaging and do not use reagents beyond their expiration date.

3. The opened kit should be stored according to the storage conditions listed in the Components Table. The shelf life of the opened kit is 30 days from the date of opening.

【Reagent Preparation】

Bring all reagents to room temperature (20-25°C) before use. If crystals are present in the solution, allow the reagents to equilibrate until the crystals are completely dissolved. If needed, incubate at 37°C for 10-15 minutes to facilitate dissolution.

According to Table 3, reconstitute the provided lyophilized product with ultrapure water to prepare the stock solution. Allow the vial to stand at room temperature for 15 to 30 minutes, then gently pipette up and down to mix. Do not vortex or shake vigorously.

Store the reconstituted stock solution at -70°C. It is recommended to aliquot the stock solution to avoid repeated freeze-thaw cycles. Do not exceed one freeze-thaw cycle. Each aliquot should contain at least 5 µg of material.

Table 2. Preparation Method

ID	Components	Size	Stock Solution Conc.	Reconstitution Buffer and Vol.
PKA002-C02	MMAF-ADC Standard	20 µg	200 µg/mL	100 µL water
PKA002-C03	HRP-Anti-Human-IgG-Fc Antibody	20 µg	200 µg/mL	100 µL water

【Assay Procedure】

1. Preparation of Working Solution

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to a final volume of 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to a final volume of 100 mL.

1.3 Preparation of HRP-Anti-Human-IgG-Fc Antibody working solution:

Dilute HRP-Anti-Human-IgG-Fc Antibody to 0.1 µg/mL with 1×Dilution Buffer. The prepared working solution should avoid light. Please prepare it for one-time use only.

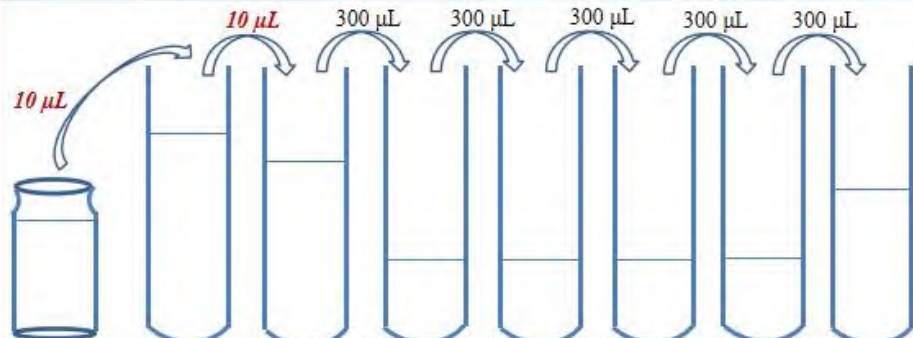
1.4 Sample preparation:

If the test sample is cynomolgus monkey serum or plasma, dilute the sample at a 1:20 dilution ratio using 1×Dilution Buffer (sample: diluent = 1:19, v/v).

2. Preparation of Standard

Prepare serial dilutions of the MMAF-ADC Standard using 1×Dilution Buffer as recommended in Figure 2.

Figure 2. Preparation of MMAF-ADC Standard

Tubes/ Solution Code	Standard stock solution	Std.-0	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6
Procedure								
Solution Conc.	200 µg/mL	2000 ng/mL	25 ng/mL	12.5 ng/mL	6.25 ng/mL	3.125 ng/mL	1.563 ng/mL	0.781 ng/mL
Dilution Buffer Vol.		990 µL	790 µL	300 µL	300 µL	300 µL	300 µL	300 µL

3. Addition of Samples

Add 100 µL of serially diluted MMAF-ADC Standard and samples to the corresponding wells. Add 100 µL of 1×Dilution Buffer to the Blank control wells. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

Note: It is recommended to run all standards and samples in duplicate wells.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 30s, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the washing step above three times.

5. Addition of HRP-Anti-Human-IgG-Fc Antibody

Add 100 µL **HRP-Anti-Human-IgG-Fc Antibody (dilute to 0.1 µg/mL)** working solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 µL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 minutes, avoid light.

8. Termination

Add 50 μ L **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing.

Note: The color in the wells will change from blue to yellow.

9. Data Recording

Measure the absorbance at 450 nm with a 630 nm reference within 5 minutes after adding the stop solution.

Note: Subtracting the OD_{630nm} value from the OD_{450nm} value helps reduce background interference.

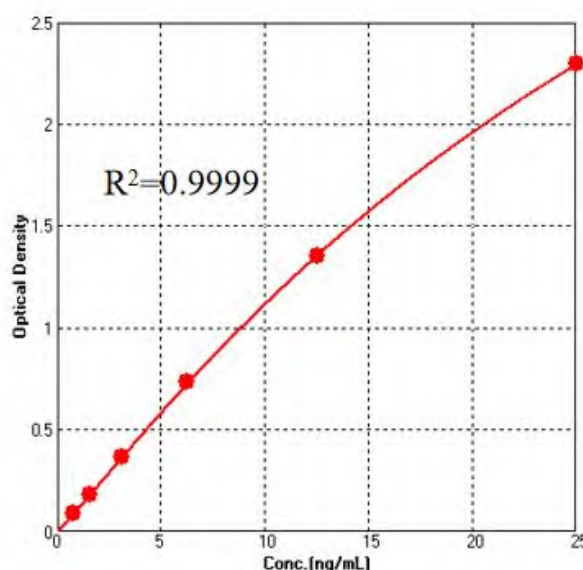
【Calculation of Results】

1. Calculate the mean absorbance of duplicate wells for each standard and sample. Subtract the absorbance of the zero standard (Blank) from each value ($OD_{450\text{ nm}} - OD_{630\text{ nm}} - \text{Blank}$).
2. Generate the standard curve by plotting the standard concentrations on the x-axis and the corrected absorbance values on the y-axis. Fit the curve using a four-parameter logistic (4-PL) model. The coefficient of determination (R^2) should be ≥ 0.9900 .
3. The assay detection range is 0.781 ng/mL-25 ng/mL.
4. If the OD value of the test sample exceeds that of the highest standard solution, dilute the sample with dilution buffer and repeat the assay.

【Typical Data】

Absolute OD values may vary depending on the laboratory, operator, and equipment used. The example data provided below is for reference only.

Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
25	2.374	2.237	2.306	2.295
12.5	1.376	1.355	1.366	1.355
6.25	0.746	0.741	0.744	0.733
3.125	0.376	0.373	0.375	0.364
1.563	0.194	0.191	0.193	0.182
0.781	0.102	0.103	0.103	0.092
0	0.011	0.010	0.014	/



【Precision】

1. Intra-assay Precision

Three samples of known concentration were tested ten replicates within a single plate to evaluate intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three independent assays to evaluate inter-assay precision.

Sample		Cynomolgus monkey serum			Cynomolgus monkey plasma		
		1	2	3	1	2	3
Intra-assay Precision (n=10)	Mean (ng/mL)	18.971	8.015	4.206	19.257	8.110	4.269
	SD	0.983	0.440	0.247	1.130	0.388	0.197
	CV (%)	5.2	5.5	5.9	5.9	4.8	4.6
Inter-assay Precision (n=3)	Mean (ng/mL)	18.722	8.372	4.029	19.164	8.116	4.236
	SD	0.711	0.480	0.172	0.321	0.078	0.085
	CV (%)	3.8	5.7	4.3	1.7	1.0	2.0

【Recovery】

Three samples with different concentrations were tested to calculate the recovery rate.

ID	Cynomolgus monkey serum		Cynomolgus monkey plasma	
	Average Recovery (%)	Range (%)	Average Recovery (%)	Range (%)
High	94.0	83.3-107.7	95.8	87.1-106.1
Middle	104.7	91.2-118.1	101.5	93.7-114.5
Low	100.7	91.1-115.3	105.9	90.0-118.2

【Linearity】

To assess the assay linearity, a high-concentration standard was spiked into different dilution matrices and serially diluted to generate concentrations within the dynamic range of the assay.

		Cynomolgus monkey serum	Cynomolgus monkey plasma
1:2	Average Recovery (%)	96.6	95.4
	Range (%)	84.7-113.2	87.2-108.7
1:4	Average Recovery (%)	99.7	94.7
	Range (%)	94.4-108.3	87.8-101.4
1:8	Average Recovery (%)	106.3	96.5
	Range (%)	95.7-117.7	89.0-103.3
1:16	Average Recovery (%)	110.7	100.7
	Range (%)	103.8-115.8	90.4-114.4

【Specificity】

Two concentrations of MMAF-ADC quality control (QC) samples were spiked with different amounts of MMAE-ADC. The results showed that MMAE-ADC did not interfere with the detection of MMAF-ADC.

MMAF-ADC Conc.(ng/mL)	MMAE-ADC Conc.(ng/mL)	Recovery (%)	CV (%)
25	5000	102.3	2.1
	500	108.6	5.6
1.563	5000	118.0	0.6
	500	119.4	1.7

【Precautions】

1. For research use only. Not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents to room temperature (20-25°C) before use. If crystals are present in the solution, allow the reagents to equilibrate until the crystals are completely dissolved. Please perform all operations in a clean environment.
5. Use before the expiration date indicated on the label.

【Troubleshooting Guide】

Problem	Cause	Solution
Low signal	a. Kit components were not equilibrated to room temperature before use; b. Insufficient reconstitution time for lyophilized components.	a. Remove the kit from 2-8 °C storage in advance and allow all reagents to fully equilibrate to room temperature before starting the assay; b. After adding reconstitution buffer, allow lyophilized components to stand for at least 15 minutes and mix gently before use.
Poor assay reproducibility	a. Improper storage of reagents after opening; b. In consistent timing during sample dilution or pipetting.	a. Store reagents strictly according to the instructions in this manual; b. Plan the experimental workflow in advance and properly schedule assay timing.
High background	a. Insufficient washing; b. Excessive incubation temperature or prolonged substrate development time; c. Reagent contamination.	a. Increase soak time during wash steps and ensure the plate is thoroughly blotted dry after final wash; b. Strictly follow the operating procedures described in the manual; c. Use clean reagents and consumables and maintain a clean experimental environment.
Edge effects	Uneven temperature distribution across the plate.	Ensure uniform incubation temperature and avoid stacking plates during incubation.
Good standard curve but no detectable signal in samples	a. Interfering substances present in the samples; b. Target analyte concentration below the assay detection.	a. Optimize sample dilution to minimize matrix interference; b. Use a higher-sensitivity assay if lower analyte levels are expected.