Anti-MMAE Antibody [PSH0-03]

HA721256

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Species independent
Applications:	ELISA
Clone number:	PSH0-03

Description: Monomethyl auristatin E is an antimitotic agent which inhibits cell division by blocking the polymerisation of tubulin. The linker to the monoclonal antibody is stable in extracellular fluid, but is cleaved by cathepsin once the conjugate has entered a tumor cell, thus activating the antimitotic mechanism. Because of its toxicity, it cannot be used as a drug itself; instead, it is linked to a monoclonal antibody (MAB) which directs it to the cancer cells. In International Nonproprietary Names for MMAE-MAB-conjugates, the name vedotin refers to MMAE plus its linking structure to the antibody. It is a potent antimitotic drug derived from peptides occurring in marine shell-less mollusc Dolabella auricularia called dolastatins which show potent activity in preclinical studies, both in vitro and in vivo, against a range of lymphomas, leukemia and solid tumors. These drugs show potency of up to 200 times that of vinblastine, another antimitotic drug used for Hodgkin lymphoma as well as other types of cancer.

Immunogen: MMAE-OVA

Recommended Dilutions:

ELISA	1:10,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

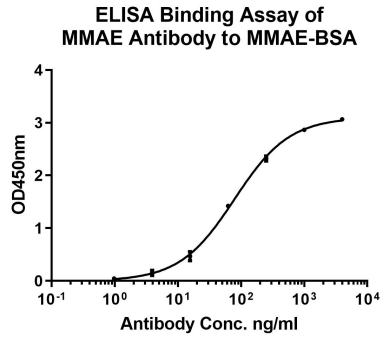

Fig1: Indirect ELISA analysis of MMAE was performed by coating wells of a 96-well plate with 50 µl per well of MMAE-BSA diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with 1%BSA blocking buffer, and incubated with 100 µl per well of MMAE monoclonal antibody (HA721256) serial diluted starting from a concentration of 20ug/ml for 1 hours at room temperature. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-Rabbit IgG secondary antibody at a dilution of 1:15,000 for one hour at room temperature. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

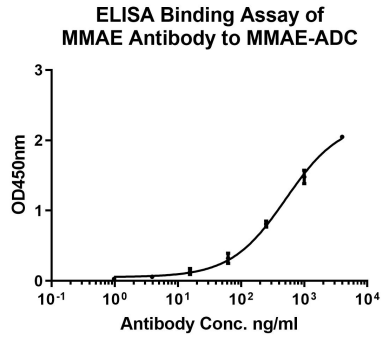

Fig2: Indirect ELISA analysis of MMAE was performed by coating wells of a 96-well plate with 50 µl per well of MMAE-ADC diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with 1%BSA blocking buffer, and incubated with 100 µl per well of MMAE monoclonal antibody (HA721256) serial diluted starting from a concentration of 20ug/ml for 1 hours at room temperature. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-Rabbit IgG secondary antibody at a dilution of 1:15,000 for one hour at room temperature. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

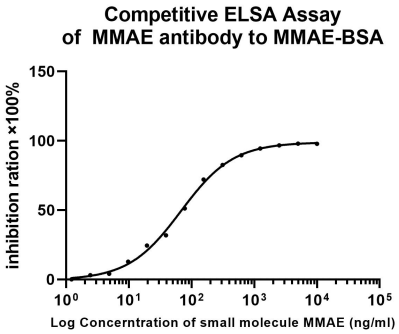

Fig3: Competitive ELISA analysis of MMAE was performed by coating wells of a 96-well plate with 50 µl per well of MMAE-BSA diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with 1%BSA blocking buffer, and incubated with 100 µl per well of MMAE monoclonal antibody (HA721256) at concentration of 1 µg/mL with serial diluted MMAE starting from a concentration of 10ug/ml for 1 hours at room temperature. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-Rabbit IgG secondary antibody at a dilution of 1:15,000 for one hour at room temperature. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

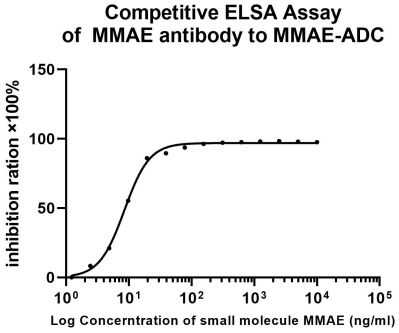

Fig4: Competitive ELISA analysis of MMAE was performed by coating wells of a 96-well plate with 50 µl per well of MMAE-ADC diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with 1%BSA blocking buffer, and incubated with 100 µl per well of MMAE monoclonal antibody (HA721256) at concentration of 1 µg/mL with serial diluted MMAE starting from a concentration of 10ug/ml for 1 hours at room temperature. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-Rabbit IgG secondary antibody at a dilution of 1:15,000 for one hour at room temperature. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

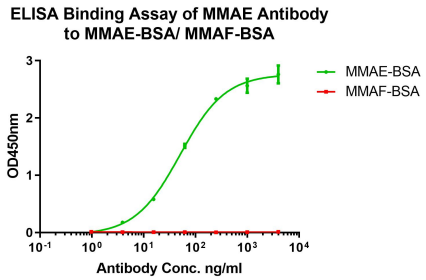

Fig5: Indirect ELISA analysis of MMAE was performed by coating wells of a 96-well plate with 50 µl per well of MMAE-BSA / MMAF-BSA diluted in carbonate/bicarbonate buffer, at a concentration of 1 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with 1%BSA blocking buffer, and incubated with 100 µl per well of MMAE monoclonal antibody (HA721256) serial diluted starting from a concentration of 20ug/ml for 1 hours at room temperature. The plate was washed and incubated with 50 µl per well of an HRP-conjugated goat anti-Rabbit IgG secondary antibody at a dilution of 1:15,000 for one hour at room temperature. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Francisco, Joseph A; et al. (2003). "cAC10-vcMMAE, an anti-CD30–monomethyl auristatin E conjugate with potent and selective antitumor activity". Blood. 102 (4): 1458–1465.
2. Dosio, F.; Brusa, P.; Cattel, L. (2011). "Immunotoxins and Anticancer Drug Conjugate Assemblies: The Role of the Linkage between Components". Toxins. 3 (12): 848–883.