MonoclonalAntibody **MABEL INC.**

MONOCLONAL ANTIBODY

Anti-5-Fluorouracil

purified with protein G affinity column

Prod. No. NM-MA-004 Lot No. 0703190010

Background	5-Fluorouracil (5-FU) is a pyrimidine analogue and inhibits an enzyme called thymidylate synthetase, which results in inhibition of DNA replication. Thus, 5-FU is used as a drug in the treatment of cancers including colorectal cancer, pancreatic cancer and skin cancer.
Specificity	5-FU (both free 5-FU and protein-bound 5-FU)
Clone	Clone H3-17 (Mouse monoclonal antibody)
Subclass	IgG1, λ
Volume	50 µl at 100 µg/ml
Source	The hybridoma was established by fusion of mouse myeloma cells with Balb/c mouse splenocytes immunized with BSA conjugated with 5-Fluorouridine. This hybridoma (Clone H3-17) was cultured in peritoneal cavity of mouse. The target antibody was concentrated in ascites of the mouse. The ascites was collected and purified with protein G affinity column. The IgG fraction was dialysed against PBS. The dialysate was then freeze.
Storage	Store at -20 to -80°C. It should be divided into small quantity to avoid many freezing and thawing. No preservative is contained.
Applications	ELISA ; 1: 100-1000 Western blotting ; Not tested Immunocytochemistry ; Not tested Immunoprecipitation ; Not tested Immunohistochemistry ; Not tested Flow cytometry ; Not tested Optimal dilutions/concentrations should be determined by an end user.

For research use only. Not for clinical diagnosis.

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Antibody characterization

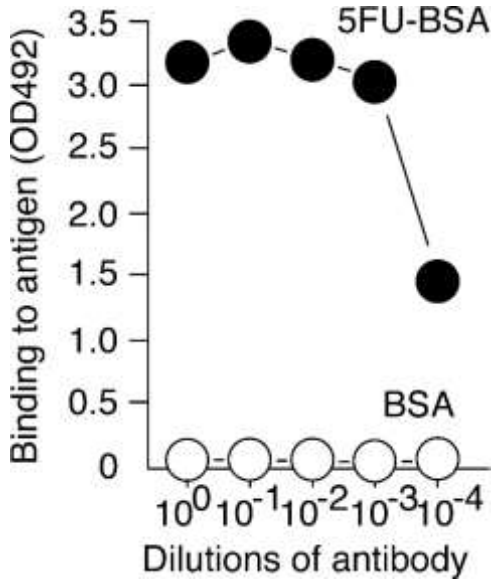

Fig. 1. Monoclonal antibody (H3-17) shows high binding to 5-FU-BSA but undetectable binding to BSA. Different dilutions of antibody were tested for binding to immobilized antigens (100 ng/well) in a direct ELISA.

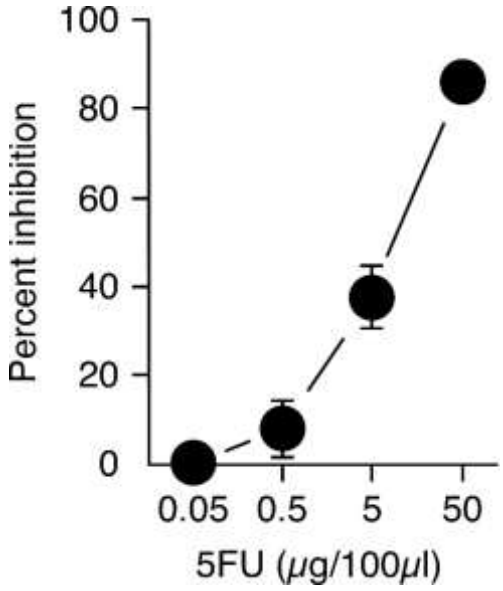

Fig. 2. Monoclonal antibody (H3-17) is capable of binding to free 5-FU. Free 5-FU efficiently inhibits the antibody binding to immobilized 5-FU-BSA, which was detected by a competitive ELISA.

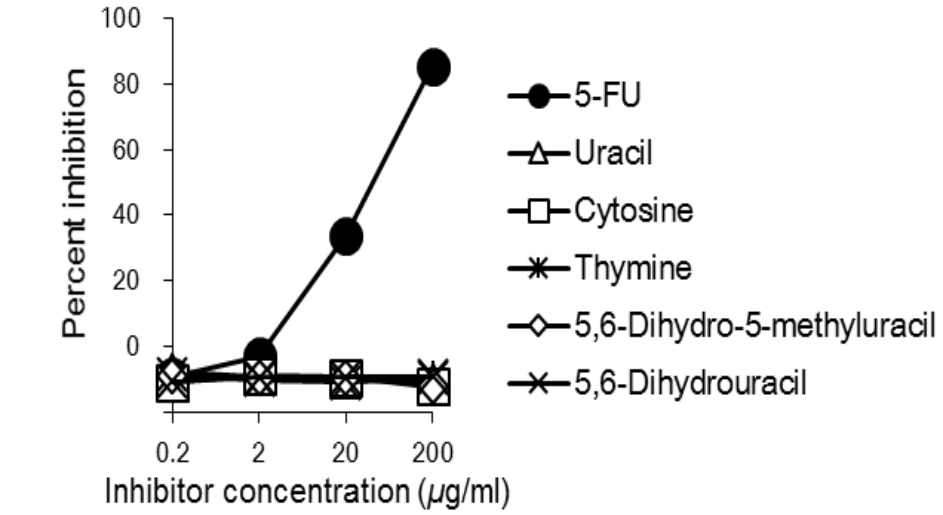

Fig. 3. Binding specificity of Anti-5-FU antibody. Anti-5-FU monoclonal antibody (H3-17) binds to 5-FU, but not to Uracil, Cytosine,Thymine,5,6-Dihydro-5-methyluracil and 5,6-Dihydrouracil.

ELISA Protocols

A. Direct ELISA (Fig. 1)

1) Prepare 5-FU-BSA solutions in PBS at the concentration of 2 μg / mL.
2) Distribute 50 μL / well of the 5-FU-BSA solution to 96 well microtiter plates.
3) Seal the plates with plate seals, and leave overnight at 4 ℃.
4) Wash the 5-FU-BSA-coated plates 5 times with 150 μL / well of PBS-T (0.05% Tween-20 in PBS).
5) Distribute 150 μL / well of 2% FBS in PBS to each well to prevent non-specific antibody binding.
6) Incubate 30 minutes at 37 ℃.
7) Wash the plates 5 times with 150 μL / well of PBS-T.
8) Prepare serial dilutions of H3-17 antibody solutions in PBS.
9) Distribute 100 uL / well of H3-17 antibodies and incubate 30 minutes at 37 ℃.
10) Wash the plates 5 times with 150 μL / well of PBS-T.
11) Distribute 100 μL / well of 1:2500 HRP-goat anti-mouse IgG (H+L) (invitrogen, Cat. No. 62-6520) diluted with PBS to each well and incubate 30 minutes at 37 ℃.
12) Wash the plates 5 times with 150 μL/ well of PBS-T.
13) Distribute 100 μL / well of the substrate solution [o-Phenylene diamine 8 mg, H_2O_2 (30%) 4 μL, Citrate-phosphate buffer (pH5.0) 20 mL] to each well and incubate 30 minutes at 37 ℃.
14) Add 50 μL / well of 2M H_2SO_4 to each well and stop enzyme reaction.
15) After gentle mixing, determine the absorbance at 492 nm of each well by a spectrophotometer.

B. Competitive ELISA (Fig. 2)

1) Prepare 5-FU-BSA solutions in PBS at the concentration of 0.1 μg / mL (5 ng / well).
2) Distribute 50 μL / well of the 5-FU-BSA solution to 96 well microtiter plates.
3) Seal the plates with plate seals, and leave overnight at 4 ℃.
4) Wash the 5-FU-BSA-coated plates 5 times with 150 μL/ well of PBS-T (0.05% Tween-20 in PBS).
5) Distribute 150 μL/ well of 2% FBS in PBS to each well to prevent non-specific antibody binding.
6) Incubate 30 minutes at 37 ℃.
7) Wash the plates 5 times with 150 μL/ well of PBS-T.
8) Prepare 5-FU (competitor, 50 uL) solutions in tubes which concentrations are 0, 0.01, 0.1, 1, 10 ug/ 50 uL PBS. Add 50 μL of 1:500 H3-17 antibody solution to each tube, which gives 50% of the maximum binding to the solid-phase antigen. And mix gently.
9) Distribute 100 uL /well of mixtures to each well and incubate 30 minutes at 37 ℃.
10) Wash the plates 5 times with 150 μL/ well of PBS-T.
11) Distribute 100 μL / well of 1:2500 HRP-goat anti-mouse IgG (H+L) (invitrogen, Cat. No. 62-6520) diluted with PBS to each well and incubate 30 minutes at 37 ℃.
12) Wash the plates 5 times with 150 μL / well of PBS-T.
13) Distribute 100 μL / well of the substrate solution [o-Phenylene diamine 8 mg, H_2O_2 (30%) 4 μL, Citrate-phosphate buffer (pH5.0) 20 mL] to each well and incubate 30 minutes at 37 ℃.
14) Add 50 μL / well of 2M H_2SO_4 to each well and stop enzyme reaction.
15) After gentle mixing, determine the absorbance at 492 nm of each well by a spectrophotometer.

Related Products

Product Name	Cat#
Anti-5-Fluorouracil (Ammonium sulphate precipitation)	NM-MA-002
5-FU-BSA (5-Fluorouracil Bovine Serum Albumin conjugate)	NM-MA-R001

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