

**MONOCLONAL ANTIBODY**

**Anti-5-Fluorouracil**

**Prod. No. NM-MA-002      Lot No. 0907290001**

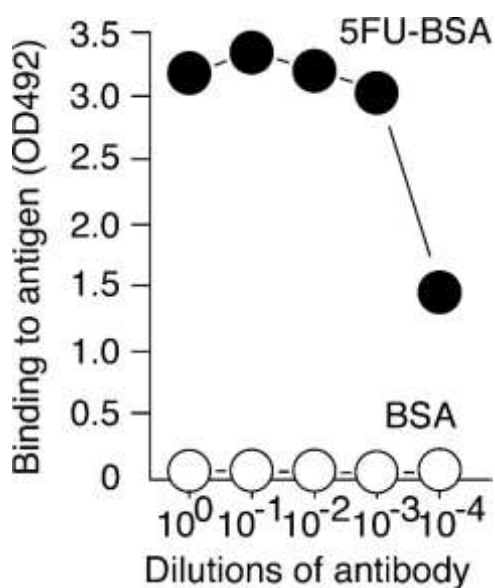
<b>Background</b>	5-Fluorouracil (5-FU) is a pyrimidine analogue and inhibits an enzyme called thymidylate synthetase, which results in inhibition of DNA replication. Thus, 5-FU is used as a drug in the treatment of cancers including colorectal cancer, pancreatic cancer and skin cancer.
<b>Specificity</b>	5-FU (both free 5-FU and protein-bound 5-FU)
<b>Clone</b>	Clone H3-17 (Mouse monoclonal antibody)
<b>Subclass</b>	IgG1, $\lambda$
<b>Volume</b>	50 $\mu$ L
<b>Source</b>	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) culture supernatant was collected and precipitated with ice-cold ammonium sulfate. After centrifugation, the pellet dissolved in small volume of double-distilled water was dialysed against PBS. The dialysate was then lyophilized.
<b>Formulation</b>	This antibody is lyophilized form. Reconstitute with 50 $\mu$ L of distilled water. No preservative is contained.
<b>Storage</b>	Lyophilized form: store at -20 to -80 °C. Reconstituted form: store at -20 °C. After reconstitution, it is stable for at least 1 year when stored at -20°C. It should be divided into small quantity to avoid many freezing and thawing.
<b>Applications</b>	ELISA ; 1: 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.
<b>Related products</b>	5FU-BSA (Cat#:MAB-Dia-5FU-BSA)

***For research use only. Not for clinical diagnosis.***

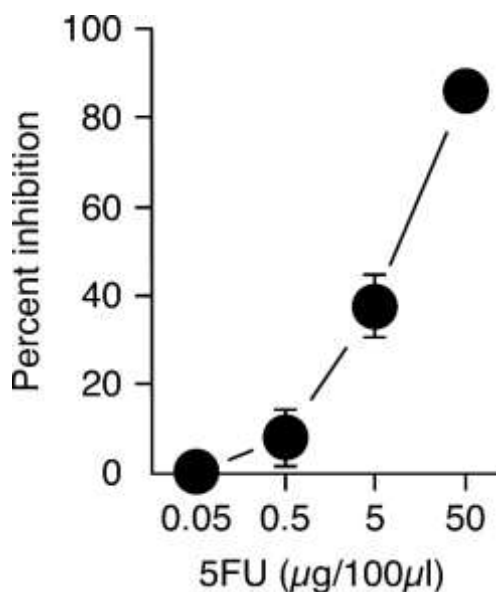
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Antibody characterization



**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.

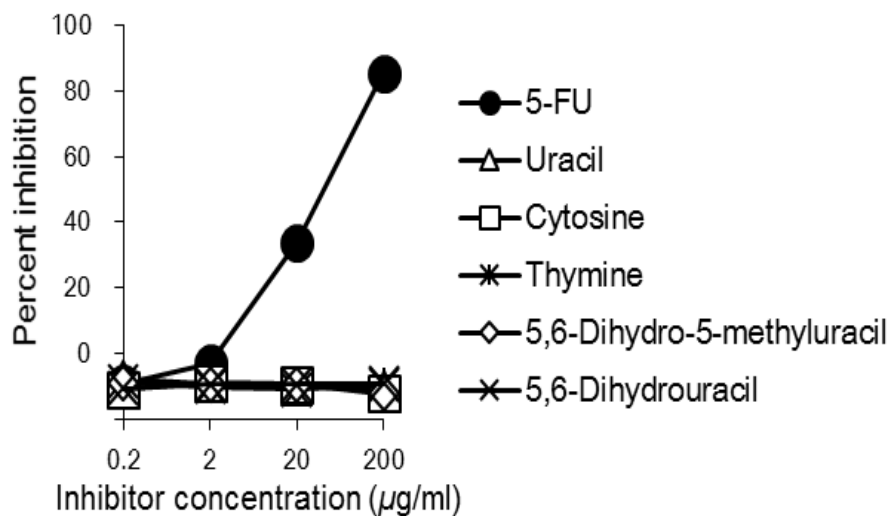


**Fig. 2. Monoclonal antibody (H3-17) is capable of binding to free 5-FU.** Free 5-FU efficiently inhibits the antibody binding to immobilized 5FU-BSA, which was detected by a competitive ELISA.

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**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 °C.
- 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 °C.
- 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 µL / well of H3-17 antibodies and incubate 30 minutes at 37 °C.
- 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 °C.
- 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<sub>2</sub>O<sub>2</sub> (30%) 4 µL, Citrate-phosphate buffer (pH5.0) 20 mL] to each well and incubate 30 minutes at 37 °C.
- 14) Add 50 µL / well of 2M H<sub>2</sub>SO<sub>4</sub> to each well and stop enzyme reaction.
- 15) After gentle mixing, determine the absorbance at 492 nm of each well by a spectrophotometer.

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**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 °C.
- 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 °C.
- 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 °C.
- 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 °C.
- 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<sub>2</sub>O<sub>2</sub> (30%) 4 µL, Citrate-phosphate buffer (pH5.0) 20 mL] to each well and incubate 30 minutes at 37 °C.
- 14) Add 50 µL / well of 2M H<sub>2</sub>SO<sub>4</sub> 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 purified with protein G affinity column	NM-MA-004
5-FU-BSA (5-Fluorouracil Bovine Serum Albumin conjugate)	NM-MA-R001

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