COSMO BIO CO., LTD.
Inspiration for Life Science

MONOCLONAL ANTIBODY

For research use only. Not for clinical diagnosis.

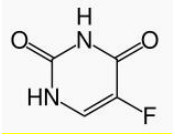
Catalog No. NM-MA-002

Anti- 5-Fluorouracil

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.

Product type	Primary antibody
Immunogen	5-Fluorouridine－BSA（5-FU-BSA）[5-FU]／[BSA]=10.2
Rased in	Mouse
Myeloma	P3-X63-Ag8.653
Clone number	H3-17
Isotype	IgG1, λ
Host	-
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) 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.
Purification	-
Form	This antibody is lyophilized form. Reconstitute with 50 µl of distilled water. No preservative is contained.
Storage buffer	PBS, No preservative is contained.
Concentration	-
Volume	50 ul
Label	Unlabeled
Specificity	5-FU (both free 5-FU and protein-bound 5-FU)
Cross reactivity	The antibody does not have cross-reactivity with Uracil, Cytosine, Thymine, 5,6-Dihydro-5-methyluracil and 5,6-Dihydrouracil.
Storage	Lyophilized form: store at -20 to -80 ℃. Reconstituted form: store at -20 ℃. After reconstitution, it is stable for at least 1 year when stored at -20 ℃. It should be divided into small quantity to avoid many freezing and thawing.
Other	< 5-Fluorouracil >



CAS.No.	51-21-8
Molecular Formula	C4H3FN2O2
Molecular Weight	130.08

Application notes ELISA

Recommended dilutions
- ELISA : 1/1000

Other applications have not been tested.
Optimal dilutions/concentrations should be determined by the end user.

References -

ANTIBODY CHARACTERIZATION

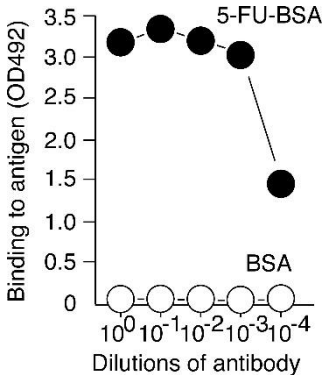


Fig.1 Monoclonal antibody (H3-17) shows high binding to 5-FU-BSA but undetectable binding to BSA. Different dilutions of antibody were tested for binding to immobilized antigens (100 ng / well) in a direct ELISA.

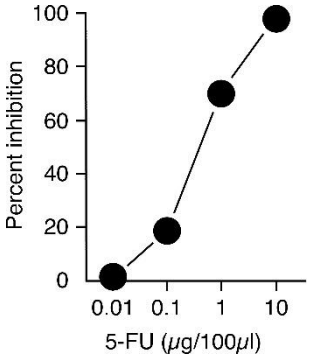


Fig.2 Monoclonal antibody (H3-17) is capable of binding to free 5-FU.. Free 5-FU efficiently inhibits the antibody binding to immobilized 5-FU-BSA (5 ng / well), which was detected by a competitive ELISA.

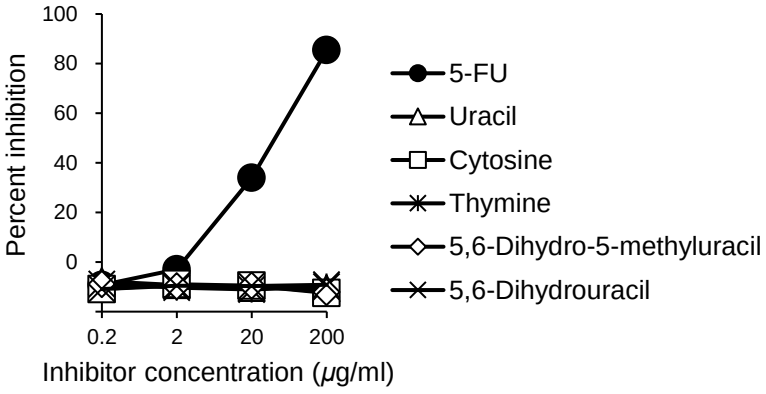


Fig.3 Binding specificity of Anti-5-FU antibody.
Anti-5-FU monoclonal antibody (H3-17) binds to 5-FU, but not to Uracil, Cytosine, Thymine,5,6-Dihydro-5-methyluracil and 5,6-Dihydrouracil.

RELATED PRODUCTS

Product Name	Maker	Cat#
5-FU-BSA (5-Fluorouracil Bovine Serum Albumin conjugate)	CSR	NM-MA-R001

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.
   Discard PBS-T, and tap the plate on the paper towel to remove the solution completely.
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 °C.
10) Wash the plates 5 times with 150 μL / well of PBS-T.
    Discard PBS-T, and tap the plate on the paper towel to remove the solution completely.
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.
    Discard PBS-T, and tap the plate on the paper towel to remove the solution completely.
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 °C.
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 °C.
4) Wash the 5-FU-BSA-coated plates 5 times with 150 μL/ well of PBS-T (0.05% Tween-20 in PBS).
   Discard PBS-T, and tap the plate on the paper towel to remove the solution completely.
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.
   Discard PBS-T, and tap the plate on the paper towel to remove the solution completely.
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. Discard PBS-T, and tap the plate on the paper towel to remove the solution completely.
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.
    Discard PBS-T, and tap the plate on the paper towel to remove the solution completely.
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 °C.
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.

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