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.

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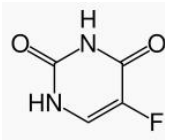
|---|---|
| **Product type** | Primary antibody |
| **Immunogen** | 5-Fluorouridine－BSA（5-FU-BSA）[5-FU]／[BSA]=10.2 |
| **Rased in** | Mouse |
| **Myeloma** | P3-X63-Ag8.653 |
| **Clone number** | H3-17 |
| **Isotype** | IgG1, λ |
| **Host** | - |
| **Source** | The hybridoma was established by fusion 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.<br>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** | - |
| **Storage** | Lyophilized form: store at -20 to -80 ℃. Reconstituted form: store at -20 ℃.<br>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 > |

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

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|---|---|
| **Application notes**<br>Recommended dilutions | ELISA<br>・ ELISA : 1/1000<br><br>Other applications have not been tested.<br>Optimal dilutions/concentrations should be determined by the end user. |
| **References** | - |

## ANTIBODY CHARACTERIZATION

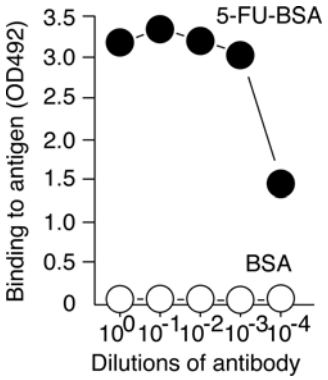


**Fig.1 Monoclonal antibody (H3-17) shows high binding to 5-FU-BSA but undetectable binding to BSA**. Different dilutions of antibody were tested for binding to immobilized antigens (100 ng / well) in a direct ELISA.

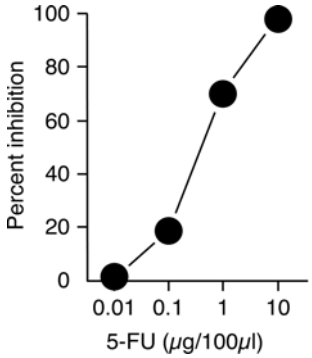


**Fig.2 Monoclonal antibody (H3-17) is capable of binding to free 5-FU.**. Free 5-FU efficiently inhibits the antibody binding to immobilized 5-FU-BSA (5 ng / well), which was detected by a competitive ELISA.

## 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.
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.
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, $H_2O_2$ (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).
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, $H_2O_2$ (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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