COSMO BIO CO., LTD.
Inspiration for Life Science

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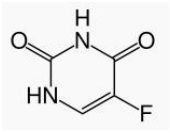
Catalog No. NM-MA-R001

5-FU-BSA
[5-Fluorouracil Bovine Serum Albumin conjugate]

Product Description	5-Fluorouracil Bovine Serum Albumin conjugate (5-FU-BSA) 5FU / BSA molar ratio 11.91.
Form	This conjugate is lyophilized form. Reconstitute with 50 µl of distilled water.
Storage buffer	PBS, No preservative is contained.
Concentration	1 mg/ml (After reconstitution).
Volume	50 ul / 1 VIAL
Storage	Lyophilized form (Before reconstitution) : store at -20°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 freezing and thawing.
Other	< 5-Fluorouracil >



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

References -

CHARACTERIZATION

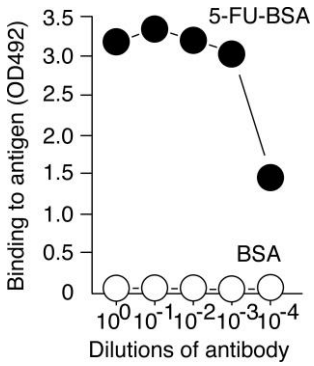


Fig.1 Monoclonal antibody (H3-17) shows high binding to 5-FU-BSA but undetectable binding to BSA. Different dilutions of antibody were tested for binding to immobilized antigens (100 ng / well) in a direct ELISA.

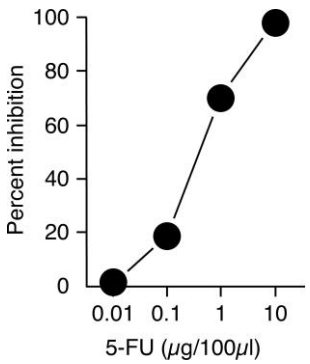


Fig.2 Monoclonal antibody (H3-17) is capable of binding to free 5-FU..Free 5-FU efficiently inhibits the antibody binding to immobilized 5-FU-BSA (5 ng / well), which was detected by a competitive ELISA.

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.

RELATED PRODUCTS

Product Name	Maker	Cat#
Anti-5-Fluorouracil	CSR	NM-MA-002

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