



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Monoclonal Anti 5-Methyl Tetrahydrofolic Acid Mouse Ascites Fluid Clone: FA-24

Product No. **M 5028**

Product Description

Monoclonal Anti 5MTH Folic Acid
(5-Methyltetrahydro FA) (mouse IgG_{2b} isotype) is presented in the form of specially processed ascites fluid obtained from BALB/c mice bearing the FA-24 hybridoma. This hybridoma is a cloned cell line derived from a fusion between a mouse myeloma cell line and splenocytes from BALB/c mice immunized with 5MTH Folic acid conjugated to KLH. The antibody is specific for an epitope present on the biologically active analog 5MTHFA but not on Folic acid. It reacts with 5MTHFA as free reagent, when conjugated to carrier such as KLH or BSA, or in the natural form in human plasma and serum when it is bound to the endogenous Folate Binder. It does not cross react with Tetrahydrofolic acid (THFA), Folinic acid (FNA), Dihydrofolic acid (FAH₂, Citrovorum Factor, Leucovorin), nor with Vitamin B₁₂. The activity of the antibody in RIA has not been tested.

Monoclonal Anti 5MTH Folic Acid is a homogenous population of antibody molecules which may be used for the detection and quantitation of 5MTHFA by Indirect and Competitive ELISA.

Vitamin B₁₂ (cobalamin) and folic acid (pteroylglutamic acid) are essential constituents for normal growth of mammalian cells. Normal range in serum is 3-16 ng/ml for folate and 0.2-0.9 ng/ml for B₁₂. Animals derive their vitamin B₁₂ only from bacterial sources; it is not found in the plant kingdom. For this reason Vitamin B₁₂ deficiency is common among strict vegetarians. Almost all animal tissues contain vitamin B₁₂. The most common form of vitamin B₁₂ deficiency, however, is the lack of Intrinsic Factor, which is necessary for vitamin B₁₂ absorption in the small intestine. This will lead to pernicious anemia.

Other less common causes of vitamin B₁₂ deficiency are abnormal intestinal bacterial flora, infestation with fish tapeworm and gastric surgery involving the ileum. Folic acid deficiency is common in pregnant women, alcoholics, those whose diets do not include raw fruits and vegetables and people with structural or functional damage to the small intestine. Vitamin B₁₂ and folic acid are metabolically interrelated. The enzyme methionine synthetase, which catalyzes the

conversion of homocysteine to methionine, requires vitamin B₁₂ and 5MTHFA as cofactors. In the absence of vitamin B₁₂, 5MTHFA cannot be converted to tetrahydrofolic acid and enter the metabolic pool of 1-carbon fragment acceptors. Since this is the only known metabolic pathway involving 5MTHFA in man, there will be a decrease in the availability of other folic acid derivatives required for miscellaneous biosynthetic pathways. One of the most important of these involves thymidylate synthetase, an enzyme necessary for DNA synthesis. Vitamin B₁₂ also functions as a cofactor in the methylmalonyl-CoA mutase reaction, which is important in both propionate and succinate metabolism. Vitamin B₁₂ and folate deficiencies are the most common causes of megaloblastic anemia, abnormal hemopoiesis, interference in the maintenance of normal nerve tissue and general intracellular uptake and function disorders in humans. Elevated levels have been encountered in hepatic and neoplastic pathologies. Vitamin B₁₂ and folate deficiencies are hematologically and clinically indistinguishable. It is therefore necessary to determine the level of vitamin B₁₂ in the serum and folate in the serum and red blood cells to establish definitively the etiology of the megaloblastic anemia, since treatment of vitamin B₁₂ deficiency with folic acid can result in serious repercussions. Levels of vitamin B₁₂ in the serum can be determined either microbiologically by time-consuming bioassays that may be affected by antimicrobial and antineoplastic agents or by competitive protein binding assays. In the binding assay natural protein binders are used, which even when they are highly purified might lead to erroneously high non-diagnostic results and necessitate the denaturing or at least the release of endogenous proteins by extreme treatments such as boiling or chemical digestion. The availability of monoclonal antibodies specific for vitamin B₁₂ or folic acid even in their bound forms provide a convenient and reliable efficient means for the simultaneous measuring of B₁₂/Folate concentration in serum or plasma both by various RIA and ELISA techniques.

The monoclonal nature of the product guarantees the continuous production of a constant titer Anti 5MTH Folic acid antibody with the same specificity and chemical identity.

Precautions

Product contains 15mM sodium azide. Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices

Product Profile

(a) Indirect ELISA: Monoclonal Anti 5MTH Folic Acid reacts specifically with 5MTH Folic acid using 5MTH Folic acid-BSA conjugate for coating of polyvinyl microtiter plate (1 µg/ml). No cross reactivity observed with Folic acid using Folic acid-BSA conjugate. Negative with vitamin B₁₂ using Vitamin B₁₂-BSA conjugate or BSA for coating.
b) Competitive ELISA: Binding of Monoclonal Anti 5MTH Folic Acid to 5MTH Folic acid-BSA coated on polyvinyl microtiter plate is inhibited by prior incubation of suboptimal dilution of the antibody with 5MTH Folic acid but not with Folic acid, Vitamin B₁₂ or by BSA.

Sensitivity

The sensitivity of competitive ELISA system has been variously defined and will depend on the methodological approach as well as on the reagents. Using a suboptimal dilution of the antibody it should be possible to detect as little as 1.0 ng/ml of 5MTH Folic Acid.

Working Dilution

The minimum antibody titer of 1:5,000 was determined by Indirect ELISA using 1 µg/ml of 5MTH Folic acid-BSA conjugate for coating of microtiter plate (procedure attached). It has been noted that 5MTH Folic acid-BSA is being absorbed most effectively on polyvinyl microtiter plates. In order to obtain best results we recommend to determine optimal working dilution by titration test.

Storage

For continuous use, store at 2-8°C. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References:

1. Chanarin, I., The Megaloblastic Anemias, Blackwell Sci. Publ., London (1969).
2. Herbert, V., in 5MTH Folic Acid, Broquist H.P. et al (Eds.), National Acad. Sci. Washington D.C., pp 227-293 (1977).
3. Halsted, C.H., Ann. Rev. Med. **31**, 79-87 (1980).

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