

Avidin from Egg White

CAS Number: 1405-69-2

Storage Temperature: Frozen

Product Number: A8280 (From Amresco 0866)

Unit definition: One unit will bind 1.0 μ g of d-biotin.

Unit activity: >10 units per mg protein.

Product Description

Appearance: White lyophilized powder

Molecular weight: approximately 66,000 Da, tetrameric

A 66 kDa glycoprotein containing four identical subunits with high binding affinity for biotin. Stable over wide pH and temperature ranges. Used in detection and protein purification methods because it can bind one molecule of biotin with each subunit. The strong avidin-biotin interaction is often utilized in affinity chromatography, ELISA, immunohistochemistry and Western Blotting.

Storage/Stability

Avidin is an extremely stable glycoprotein, and if stored frozen, should retain activity for six years.

Solubility/Solution Stability:

Avidin is very soluble in water, up to 20 mg/mL, and in salt solutions and is stable over a wide range of pH and temperatures, particularly when combined with biotin. It can be crystallized from ammonium sulfate at > 2.5 M at pH 5. Since one tryptophan residue per subunit is involved in the binding site, avidin can be inactivated by oxidizing agents such as ozone, peroxide or strong light. Solutions should be stored at -20°C.

The avidin-biotin complex is even more heat stable than avidin alone, only 10% dissociated after 15 minutes at 100°C, not completely dissociated after 60 minutes at 100°C, but only under autoclaving conditions (120°C, 15 minutes) can the complex be quantitatively dissociated. When avidin was reduced in the presence of 9 M urea, its biotin-binding activity was unchanged. The protein was denatured and lost biotin-binding activity as the pH was gradually lowered to pH 1. However, when the pH was raised to pH 3, avidin regained native configuration and binding activity. The complex is also extremely stable at high pH, being only 20% ionized even at pH 13.

General Remarks:

Some years ago it was discovered that feeding large quantities of dried egg white to animals produced a nutritional deficiency, which was treated by administration of vitamin H. Eventually it was determined that this deficiency was caused by vitamin H- also called biotin- being bound by a protein in egg white, soon called avidin, due to its "avidity" for biotin. The avidin-biotin association constant ($K=10^{15}$) is one of the strongest affinities known. This complex is therefore an extremely important tool in immunochemistry. Avidin is stable to about 85°C without biotin, but the complex is stable to 100°C, and significantly stable to detergents and denaturants.

Because of the stability of both avidin and biotin, each of these molecules has been used as "labels" for antibodies, fluorescent dyes, proteins and other molecules of interest to biochemists. Each has been incorporated into immobilized matrices; in fact, the only way monomeric avidin can exist is through its attachment to an agarose.