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Product Information

Avidin from egg white
BioUltra

Catalog Number **A9275**
Storage Temperature 2–8 °C

CAS RN 1405-69-2

Product Description

In the late 19th century and the 1910's, several reports indicated that feeding large quantities of dried egg white to animals produced a nutritional deficiency.[1,2] Administration of vitamin H, also known as biotin, remedied this deficiency. Eventually, it emerged that this deficiency resulted from the binding of biotin to a protein in egg white.[3,4] This protein was called "avidin", after its "avidity" for biotin.

Avidin is a tetrameric glycoprotein with an approximate molecular mass of 66–67 kDa.[5] It is composed of four subunits with each subunit containing 128 identical amino acid residues and a variable carbohydrate moiety.[5,6] The subunits may vary slightly in molecular mass due to the carbohydrate composition. Only Asp^{17} is glycosylated.[5,7] The carbohydrate moiety can have at least three different carbohydrate structural types.[8] Avidin can be dissociated into subunits under strongly denaturing conditions. Each subunit is separately capable of binding biotin with a dissociation constant (K_d) = 10^{-7} M.[6,9]

The avidin-biotin association constant (K_a = 10^{15} M^{-1}) is one of the strongest affinities known. The complex is stable to 100 °C, and significantly stable to detergents and denaturants.[10] Avidin is stable to ~85 °C without biotin. Biophysical studies of the avidin-biotin complex have implicated particular tryptophan and lysine residues in the biotin-binding site of avidin.[11,12] The crystal structure of avidin has been published.[13] The crystal structure of a deglycosylated form of avidin complexed with biotin has been reported.[14]

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. Avidin and biotin have each been incorporated into immobilized matrices. (The only way that monomeric avidin can exist is through its attachment to an agarose support.[9])

This product (A9275) is purified using affinity chromatography. It is dialyzed extensively against deionized water before being lyophilized. The basic procedures are modified from literature preparations.[13,15,16] This product is sold by protein content (determined by $E^{1\%}_{280}$).

Unit Definition: One unit will bind 1.0 μg of d-biotin.

Unit activity: ≥10 units per mg protein

Isoelectric point (pI):[8,9] 10

K_d for the avidin-biotin complex:[6] 10^{-15} M (neutral pH)

Binding capacity: For tetrameric avidin, the theoretical maximum is 4 moles biotin:1 mole avidin[6,13] or ~15 μg biotin/mg protein.[14]

Reported fluorescent wavelength:[9]
338 nm (avidin)
328 nm (avidin-biotin complex)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Avidin is very soluble both in water, up to 20 mg/mL,[8] and in salt solutions. Avidin solutions are stable over a wide range of pH and temperatures, particularly when combined with biotin.[6,17] Avidin can be crystallized from ammonium sulfate at >2.5 M at pH 5.[13] 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.[11] Solutions should be stored at –20 °C.

Storage/Stability

The avidin-biotin complex is even more heat stable than avidin alone:

- It is only 10% dissociated after 15 minutes at 100 °C.[6] It is not completely dissociated after 60 minutes at 100 °C.
- The complex can be quantitatively dissociated only under autoclaving conditions, e.g. 120 °C, 15 minutes.[15]

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.[17]

References

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