



## Product Information

**AVIDIN**  
from Egg White  
Sigma Prod. Nos. A9390 and A9275

**CAS NUMBER:** 1405-69-2

**UNIT DEFINITION:** One unit will bind 1.0 µg of d-biotin. Product sold by protein content (determined by  $E^{1\%}_{280}$ ).

**Unit activity:** 10-15 units per mg protein.

### PHYSICAL DESCRIPTION:

Appearance: white lyophilized powder<sup>1</sup>

Molecular weight: approximately 66,000 Da, tetrameric<sup>2</sup>

Structure: Avidin is a tetrameric glycoprotein composed of four identical subunits. Each subunit has 128 amino acid residues (from sequence analysis) and a carbohydrate moiety (MW 14,328 + 1640 = 15,968).<sup>3,4</sup>

Avidin has at least three different carbohydrate structures possible which are indistinguishable by biotin-binding, N-terminal analysis, SDS-PAGE.<sup>5</sup> Only the asparagine-17 is glycosylated.<sup>3,6</sup>

Isoelectric point (pI):  $10^{6,7}$

Dissociation constant ( $K_d$ ) for avidin-biotin complex:  $10^{-15}$  M at neutral pH<sup>4</sup>

Binding capacity: For tetrameric avidin, the theoretical maximum is 4 moles biotin: 1 mole avidin<sup>4,8</sup> or up to 15 µg biotin/mg protein.<sup>10</sup>

Avidin can be dissociated into subunits under strongly dissociating conditions. Each subunit is separately capable of binding biotin, but the  $K_d = 10^{-7}$  M.<sup>4,7</sup>

Reported fluorescent wavelength: 338 nm for avidin, 328 nm for avidin-biotin complex<sup>7</sup>

### STABILITY / STORAGE AS SUPPLIED:

Avidin is an extremely stable glycoprotein, and if stored dry at 2-8°C, should retain activity for six years.

### SOLUBILITY / SOLUTION STABILITY:

Avidin is very soluble in water, up to 20 mg/mL<sup>8</sup>, and in salt solutions and is stable over a wide range of pH and temperatures, particularly when combined with biotin.<sup>4,9</sup> It can be crystallized from ammonium sulfate at > 2.5 M at pH 5.<sup>8</sup> 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.<sup>11</sup> Solutions should be stored at -20°C.

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**SOLUBILITY / SOLUTION STABILITY:** (continued)

The avidin-biotin complex is even more heat stable than avidin alone, only 10% dissociated after 15 minutes at 100°C<sup>4</sup>, not completely dissociated after 60 minutes at 100°C, but only under autoclaving conditions (120°C, 15 minutes) can the complex be quantitatively dissociated.<sup>10</sup> 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.<sup>9</sup>

**METHOD OF PREPARATION:**

Both A9390 and A9275 are prepared from egg white from domestic chicken, but differ slightly in method of isolation. A9390 is purified by ion-exchange chromatography; A9275 is purified using affinity chromatography to the same degree of purity. Both products are dialyzed extensively against deionized water before being lyophilized. The basic procedures are modified from literature preparations.<sup>8,10,12</sup>

**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.<sup>13</sup>

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.<sup>7</sup> Sigma offers a wide variety of avidin- and biotin-conjugates, as well as closely related streptavidin (having similar affinity for biotin, but lower background staining since it contains no carbohydrate)<sup>7</sup> and Extravidin<sup>TM</sup>, a modified avidin with lower background and higher specificity. Please consult both research listings and the immunochemical section of the Sigma general catalog for available products.

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**GENERAL REMARKS:**

A vast body of research literature is based on the avidin-biotin complex; a short list is offered under ADDITIONAL REFERENCES. No attempt is made to select any one usage protocol.

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