

# Technology



# **Biotin-Binding Proteins**

The interaction between avidin (or streptavidin) and biotin exhibits one of the highest known non-covalent interactions (Fig.1). Avidin, streptavidin, monomeric avidin, and their analogs have become powerful tools for probes and affinity ligands for various biochemical assays, diagnosis, affinity purification, and drug delivery applications.



Interaction between biotin and biotin-binding protein

### Biotin

Biotin, also called vitamin H, exhibits an extraordinary binding affinity with avidin ( $Ka=10^{15}$ ) and streptavidin ( $Ka=10^{14}$ ). Such high affinity makes biotinylated moieties (proteins or other molecules) beneficial for non-radioactive detection and purification of target molecules. Biotinylating, also called biotin labeling, is covalently attaching biotin to proteins, nucleic acids, carbohydrates, or other molecules through enzymatic or chemical methods.

### Avidin

Avidin is a biotin-binding glycosylated protein derived from chicken white. It comprises four 128 amino acid subunits, each binding one biotin molecule over a wide range of pH and temperature. Since avidin can be chemically modified with little to no effect on its function and is easily isolated from chicken egg whites, it helps detect biotinylated molecules in various conditions.

### Streptavidin

Streptavidin is a tetrameric biotin-binding protein that originates from *Streptomyces avidin* and has a mass of 60 kDa. It has no carbohydrate and lower pI, giving a lower degree of nonspecific binding, making streptavidin an ideal reagent choice for many detection systems.

### **Monomeric Avidin**

Monomeric avidin, derived from the native tetrameric protein, retains the same biotin-binding specificity as native avidin, but its biotin-binding affinity dramatically decreases ( $kD = \sim 10^{-8}$  M). Therefore, the bound biotinylated molecules can be easily eluted from the monomeric avidin by mild elution conditions such as 2mM biotin-containing buffer.

### Advantages and benefits of using biotin-binding protein systems.

#### High affinity

Both streptavidin and avidin are homo-tetramers and have an extremely high biotin-binding affinity, with a  $K_D \sim 10^{-14}$  M.

• High stability

The biotin-binding complex is exceptional stability and can resist Extreme pH, temperature (2 °C and 40 °C), harsh organic solvents, denaturing agents such as guanidinium chloride, detergents such as SDS and proteolytic enzymes.

### Outstanding selectivity and specificity

The biotin and streptavidin or avidin interaction are highly specific, ensuring low nonspecific binding.



### High sensitivity

High stability and specificity ensure high detection sensitivity.

### Very flexibility

The small size and remarkable stability of biotin are exceptionally suitable for relatively easy incorporation into various molecules or specific locations in molecules with minimal perturbation to the structure and function of the conjugated biomolecules.

### Applications

Compared to other non-covalent interactions, the biotin-binding system provides tremendous benefits. It has become a versatile platform for amplifying weak signals, flow cytometry, Western blotting, ELISA, FLISA, IHC, immunofluorescence microscopy, etc. However, currently, the applications of the biotin-binding systems are mainly based on the traditional affinity chromatography matrices such as beaded agarose resin or column. Their procedures are tedious, time-consuming, and unable to handle tiny samples such as cancer-cell targeting and single-cell analysis. We developed an extremely efficient magnetic affinity system to overcome these limitations.

Magnetic beads (particles) are an entirely different type of solid support matrices from beaded agarose or other porous resins. They are much smaller (typically 1-5 µm diameter), thus providing larger surface areas for a high density of ligand immobilization. The beads are manufactured using nanometer-scale superparamagnetic iron oxide as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide. The pure inert silica makes less nonspecific binding.

### 1. BcMag<sup>™</sup> Streptavidin Magnetic Beads

The Magnetic particle is a highly uniform and superparamagnetic microsphere coated with a high density of high purity (>97%) streptavidin on the surface. The beads are manufactured using nanometer-scale superparamagnetic iron oxide as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide. The microspheres are specifically designed and tested for applications in immunoprecipitation, cell sorting, and rapid single-step capture of the target molecules such as DNA, RNA, antibody, or protein from cell lysates or hybridization reactions.

### Learn more:

### Streptavidin Magnetic Beads

### 2. BcMag<sup>TM</sup> Cleavable Streptavidin Magnetic Beads

BcMag<sup>TM</sup> Cleavable Streptavidin Magnetic Beads are uniform superparamagnetic microspheres coated with high purity of (>97%) streptavidin. Because streptavidin is linked to the beads (particles) via a cleavable disulfide linker, reducing agents such as DTT or - mercaptoethanol can cleave and separate the biotinylated molecule-streptavidin complex from the beads rather than using harsh elution reagents such as 8M guanidine or SDS detergent after affinity purification (Fig.2). The microspheres have been specifically designed and evaluated for use in immunoprecipitation, cell sorting, and quick single-step capture of biotinylated molecules from cells such as DNA, RNA, antibodies, or proteins.



Learn more:

Fig.2. Cleavable Streptavidin Magnetic Beads

Cleavable Streptavidin Magnetic Beads

### 3. BcMag<sup>™</sup> Streptavidin Terbium Fluorescent Magnetic Beads



The green, fluorescent magnetic resin is highly fluorescent, uniform, and superparamagnetic microspheres coated with streptavidin (Fig.3). The microspheres combine all the advantages of a unique streptavidin biotin-binding system, time-resolved fluorescent dyes, and magnetic properties to perform highly sensitive assays. The beads are manufactured using nanometer-scale superparamagnetic iron oxide and terbium metal as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide and terbium metal.



Fig.3. Streptavidin Terbium Fluorescent Magnetic Beads

### Learn more:

Streptavidin Terbium Fluorescent Magnetic Beads

### 4. BcMag<sup>™</sup> Streptavidin Europium Fluorescent Magnetic Beads

The red fluorescent magnetic beads are highly fluorescent and uniform superparamagnetic microspheres coated with streptavidin (Fig.4). The microspheres combine all the advantages of a unique streptavidin biotin-binding system, time-resolved fluorescent dyes, and magnetic properties to perform highly sensitive assays. The intensely red fluorescent microspheres (particles) are manufactured using nanometer-scale iron oxide and europium metal as core and entirely encapsulated by a high purity silica shell, typically ensuring no leaching problems with the iron oxide and terbium metal.



#### Learn more:

Fig.4. Streptavidin Europium Fluorescent Magnetic Beads

Streptavidin Europium Fluorescent Magnetic Beads

### 5. BcMag<sup>™</sup> Monomer Avidin Magnetic Beads

The unique magnetic beads are highly uniform superparamagnetic microspheres coated with a high density of high purity (>97%) avidin subunit monomer on the surface. Monomeric avidin, derived from the native tetrameric protein, retains the same biotin-binding specificity as native avidin, but its biotin-binding affinity dramatically decreases ( $kD = ~10^{-8}$  M). Therefore, the bound molecules can be easily eluted from the monomeric avidin beads by mild elution conditions such as 2mM biotin instead of harsh elution reagents such as 8M guanidine or SDS detergent (Fig.5). The beads perfectly fit into applications such as immunoprecipitation, cell sorting, and rapid single-step capture of biotinylated molecules such as DNA, RNA, antibody, or protein from cell lysates or hybridization reactions.





Learn more: Monomer Avidin Magnetic Beads

6. BcMag<sup>TM</sup> Avidin Terbium Fluorescent Magnetic Beads



# Technology

The super-bright green, fluorescent magnetic resin is a highly fluorescent, uniform, and superparamagnetic microsphere coated with avidin. The microspheres combine all the advantages of a unique avidin biotin-binding system, time-resolved fluorescent dyes, and magnetic properties to perform highly sensitive assays. The intensely green, fluorescent beads (particles) are manufactured using nanometer-scale superparamagnetic iron oxide and Terbium as core and entirely encapsulated by a high purity silica shell, typically ensuring no leaching problems with the iron oxide and terbium metal.

### Learn more:

Avidin Terbium Fluorescent Magnetic Beads

### 7. BcMag<sup>TM</sup> Avidin Europium Fluorescent Magnetic Beads

The super bright red fluorescent magnetic beads are highly fluorescent uniform superparamagnetic microspheres coated with a high density of avidin. The microspheres combine all the advantages of a unique avidin biotin-binding system, time-resolved fluorescent dyes, and magnetic properties to perform highly sensitive assays. The beads are manufactured using nanometer-scale superparamagnetic iron oxide and Terbium as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide and terbium metal.

### Learn more:

Avidin Europium Fluorescent Magnetic Beads

### 8. BcMag<sup>™</sup> Avidin Ruthenium Fluorescent Magnetic Beads

The Beads are highly fluorescent uniform superparamagnetic microspheres coated with a high density of avidin. The microspheres combine all the advantages of a unique avidin biotin-binding system, time-resolved fluorescent dyes, and magnetic properties to perform highly sensitive assays (Fig.6). The beads are manufactured using nanometer-scale superparamagnetic iron oxide and ruthenium metal as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide and terbium metal.



Fig.6. BcMag<sup>TM</sup> Avidin Ruthenium Fluorescent Magnetic Beads

#### Learn more:

Avidin Ruthenium Fluorescent Magnetic Beads

#### 9. BcMag<sup>TM</sup> Streptavidin Ruthenium Fluorescent Magnetic Beads

BcMag<sup>TM</sup> Streptavidin Ruthenium Fluorescent Magnetic Beads are highly fluorescent, uniform, and superparamagnetic microspheres coated with streptavidin protein. The microspheres combine all the advantages of a unique avidin biotin-binding system, time-resolved fluorescent dyes, and magnetic properties to perform highly sensitive assays (Fig.6). The beads are manufactured using nanometer-scale superparamagnetic iron oxide and ruthenium metal as core and entirely encapsulated by a high purity silica shell, ensuring no leaching problems with the iron oxide and terbium metal.

### Learn more:

Streptavidin-Ruthenium Fluorescent Magnetic Beads

### 10. His-tagged recombinant streptavidin



The highly pure (>95%) recombinant streptavidin protein with 6x His-tag at N-terminus was produced in E.coli and purified by Ni resin. It is a single, non-glycosylated polypeptide chain and has a molecular mass of 18 kDa. The recombinant streptavidin is widely used to detect biotinylated biomolecules or as probes in various applications such as Western blotting, ELISA, immunohistochemistry, and fluorescence imaging.

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