Streptavidin Particles Technical Information

Streptavidin

Streptavidin is a protein (MW of approx. 66,000) made up of four identical subunits, each containing a high affinity binding site for biotin (KD = 10-15 M). It has the same biotin binding properties as avidin, but less non-specific binding is observed.

PRINCIPLE

Streptavidin particles are designed as a matrix for simple and efficient separations of biotinylated compounds such as proteins, immunoglobulins, sugars, lectins or DNA/RNA.

INSTRUCTIONS FOR USE

Preparations of Absolute Mag™ Streptavidin Particles

The particles should be washed before use to remove the 0.02% NaN3 added as a preservative. The washing procedure is facilitated by using centrifugation for larger particles.

1. Resuspend by gently shaking the vial to obtain a homogeneous suspension.
2. Add the appropriate amount (see section on Binding Capacity below) of particles to a tube. 3. Place the tube in a centrifuge at 1.5K for 20 minutes.
4. Remove the supernatant by aspiration with a pipette. Avoid touching the particle cake with the pipette tip.
5. Add the recommended buffer along. Use the same volume as in step 2 above and resuspend gently.
6. Repeat steps 3 to 5 and after the last wash add a suitable volume of the recommended buffer to obtain an appropriate working concentration of particles.

Preparation of Absolute Mag™ Streptavidin Particles for RNA manipulations.

NOTE:Absolute Mag™ Streptavidin are NOT supplied in RNase free solution.

1. Add DEPC to a final concentration of 0.1% (1ml/l) to Solution A and Solution B (see section on Buffers and Solutions below).
2. Shake vigorously.
3. Incubate at room temperature for 1 hour and autoclave the solutions.
4. Wash the Streptavidin particles twice with the same volume of Solution A for 1-3 minutes.
5. Wash the Streptavidin particles once with the same volume of Solution B.
6. Resuspend the Streptavidin particles in Solution B.

BIOTINYLATION PROCEDURES

Biotinylated nucleic acids and proteins easily bind to Absolute Mag™ Streptavidin Particles. Following the binding procedure, the biotinylated product is maneuvered easily due to the unique magnetic properties of the streptavidin particles and the strength and stability of the biotin/streptavidin linking system. (KD = 10^{-15} M). Because of simplified handling, experimental procedures, such as changing of buffers, are easily accomplished.

E-mail: info@creative-diagnostics.com
www.cd-bioparticles.com

NOTES:

- All biotin reagents should contain a spacer arm, at least 6 C-atoms in length, to reduce steric hindrance.
- Biotinylated oligonucleotide primers should be purified by reverse phase HPLC/FPLC® chromatography for optimal binding efficiency.
- Specific biotinylation in the 5'-end of oligonucleotide primers is recommended to maintain the 3'-end free for elongation.

I. Biotinylation of oligonucleotide primers.

a) Biotinylated oligonucleotides are commercially available from a number of companies.

b) It is recommendable to directly incorporate biotin at the 5'-end of the oligo during DNA synthesis using biotin phosphoramidite. The use of biotin phosphoramidite in the synthesis reaction allows biotinylation at the 5'-end with no effect on the specificity or melting temperature of the labeled oligonucleotide.

c) Biotinylation by chemical incorporation of 5'- or 3'-aminomodified oligonucleotides:

1. Dissolve 0.1 µmol of amino-modified nucleic acid in 0.7 ml of sterile distilled water (5'-amino modified oligonucleotides are synthesized using the reagent Aminolink 2^{TM}.
2. Add 0.1 ml of 1.0 M $NaHCO_3/Na_2CO_3$ buffer, pH 9.0.
3. Attach a biotin residue to the amino group using the reagent Biotin-X-NHS-Ester. Freshly prepare a 10 mg/ml solution of Biotin-X-NHS-Ester in N,N-dimethylformamide. Add 0.2 ml of this solution in the reaction mixture.
4. Leave at room temperature for two hours.
5. Remove traces of unincorporated Biotin-X-NHS-Ester by using a NAPTM-10 Spin Column. 6. Purify the biotinylated oligonucleotide by reverse HPLC following the manufacturer's specifications.

II. Purification of biotinylated primers.

a) It is of great importance that the biotinylated oligonucleotide is pure from unbound biotin, preferably by FPLC or reverse phase HPLC, since free biotin will occupy binding sites on the beads and reduce the binding capacity of biotinylated PCR products. This purification step also ensures full length deoxyoligonucleotides with labeling levels close to 100%.

b) When performing the biotinylation of primers using biotin phosphoramidite, you can expect to obtain 70-75% biotinylation of your primer. This means that 25-30% of your primer may be non-biotinylated. Reverse phase HPLC or FPLC is necessary to recover the biotinylated primers in pure form.

III. Biotinylation of already synthesised oligonucleotides.

The Absolut Mag™ Labelling system makes it possible to introduce biotin into already synthesized oligos. The biotin is randomly incorporated in the oligonucleotide.

IV. Biotinylation of larger DNA fragments.

a) End-labeling using PCR with a biotinylated primer.

E-mail: info@creative-diagnostics.com
www.cd-bioparticles.com

b) Enzymatic incorporation of a biotin dUTP label. A biotin dUTP label can be incorporated enzymatically into a double-stranded DNA fragment through end-labeling by use of Klenow DNA polymerase enzyme, nick translation or mixed primer labeling.
c) Photobiotinylation. The photoactivated form of biotin can be incorporated randomly in the DNA fragment with UV light.

V. Biotinylation using cleavable reagents.

a) Enzymatic incorporation of a biotin dUTP analogue with a cleavable linker. Incorporation of a biotin with a linker arm containing a disulphide bond allows for a simple dissociation of the DNA fragment, as the disulphide links easily become cleaved with dithiothreitol (DTT). This reagent is enzymatically incorporated into a DNA fragment either by end-labeling, nick translation or mixed primer labeling. Biotin -21-SS-dUTP is recommended.
b) Chemical incorporation of the guanido analogue of NHS biotin. Incorporation of iminobiotin allows for the dissociation of the bound nucleic acid fragment with a simple pH change. The streptavidin/iminobiotin complex is dissociated at pH 4.0. At pH 9.5 or greater, iminobiotin will bind tightly to Absolute Mag™ Streptavidin particles. The released iminobiotin can be re-immobilised onto Absolute Mag™ Streptavidin particles.

VI. Biotinylation of amino-modified DNA.

Amino-modified nucleic acid fragments can be chemically biotinylated using a biotin-X-NHS Ester.

VII. Biotinylation of RNA fragments.

In most cases, the same biotinylation procedure will work for both DNA and RNA. Already synthesised RNA fragments can be photobiotinylated. As with DNA, the photoactivated forms of biotin randomly incorporate into the RNA fragment with UV light.

VIII. Biotinylation of proteins.

Proteins can be chemically biotinylated using a biotin-X-NHS ester.
Example of antibody biotinylation:

1. Calculate the number of purified antibody molecules per volume unit.
2. Dissolve 10 X molar excess of the biotinylation reagent Biotin-X-NHS Ester (MW = 454.5) in 10 µl DMSO and add this solution to the antibody solution.
3. Add the required amount of a 1.0 M NaHCO3 stock solution, pH 8.0 to obtain a final concentration of 0.1 M. Check pH and adjust to 8.0 if necessary.
4. Incubate overnight at 4°C.
5. Filter on a gel, e.g. Biogel® P-30 in PBS with 0.1 M NaN3 (final conc.)
6. Calculate the final concentration of antibodies and store at 4°C. A final concentration of 0.1% BSA or similar should be added.

IX. Cleavable biotin derivatives of proteins.

NHS-biotin containing a cleavable disulphide bond allows for the easy cleavage of the desired protein from the biotin/streptavidin complex.

IMMOBILISATION PROCEDURES

I. Nucleic acids

1. Wash the Absolute Mag™ Streptavidin particles once in the B&W Buffer as described below (see "Buffers and Solutions").
2. Aliquot in microcentrifuge tubes or microtiter wells and remove the buffer from the last washing step.
3. Resuspend the beads in B&W Buffer to a final concentration of 5 µg/µl (twice the original volume), or to a concentration suitable for the application of choice.
4. To immobilise, add an equal volume of the biotinylated DNA/RNA. The NaCI concentration in the B&W Buffer is 2 M and the final NaCI concentration in the binding mixture should be 1 M. The amount of DNA/RNA needed is dependent on the application.
5. Incubate at room temperature using gentle rotation or occasional mixing by gently tapping the tubes. The optimal incubation time depends on the length of the nucleic acid bound: short oligonucleotides (less than 30 bases) required at most 10 minutes. DNA fragments up to 1 kb require 15 minutes.
6. Separate the beads, now coated with the biotinylated DNA/RNA fragment, by centrifugation.
7. Wash 2-3 times with a 1 x B&W Buffer (the buffer suggested below is 2 x concentrated), using centrifugation.
8. Resuspend to the desired concentration. The binding is now complete and the beads with the immobilised DNA/RNA fragment can be resuspended in a buffer with low salt concentration, suitable for downstream applications.

II. Antibodies.

1. Calculate the amount of biotinylated antibodies needed. Between 5-10 µg antibodies per mg Absolute Mag™ Streptavidin particles is sufficient when saturation of the streptavidin is desired, assuming 100% biotinylation of the antibody. Check the binding capacity for antibody per mg
2. Incubate at room temperature for 30 minutes with gentle rotation of the tube.
3. Separate the Streptavidin particles now coated with biotinylated antibodies using centrifugation.
4. Wash 4-5 times in PBS/BSA.
5. Resuspend to the desired concentration.

BINDING CAPACITY OF ABSOLUTE MAG™ STREPTAVIDIN

The binding capacity of Absolute Mag™ Streptavidin particles is fragment length dependent. Quantitative assays of 32Plabelled PCR product binding to beads show the effect of the length of the PCR product on binding. Twice as many copies of a 500 bp DNA fragment bind than a 1,000 bp DNA fragment. Reduced binding capacity for large DNA fragments may be due to steric hindrance.

The salt concentrations influence the efficiency of the binding of biotinylated nucleic acids to Absolute Mag™ Streptavidin particles. Optimal binding conditions for biotinylated DNA fragments onto Absolute Mag™ Streptavidin particles:

Up to 1kb 1M NaCI (final concentration)

25°C

15 minutes

Because free biotin and free biotinylated oligonucleotides (not used during a PCR amplification) bind to the beads much more rapidly than longer PCR fragments, it is most important to ensure that the solution containing the PCR fragments does not contain an excess of these components. To prevent an excess of free biotinylated oligonucleotide, one may perform PCR with limiting concentrations of the biotinylated primer, or remove the free biotinylated primer via precipitation or by microdialysis.

One milligram of Absolute Mag™ Streptavidin particles typically binds:

- 700 pmoles of free biotin.
- 200 pmoles of biotinylated oligonucleotides (single stranded).
- 5 - 10 µg of biotinylated antibody.
- 5 pmoles of a 2-4 kb double stranded DNA fragment.

NOTE:

CD recommends that a titration be performed to optimize the quantity of beads used for each individual application since both fragment size and biotinylation procedures will affect the binding capacity of the beads.

Buffers and Solutions

Solution A: DEPC-treated 0.1 M NaOH, DEPC-treated 0.05 M NaCl

Solution B: DEPC-treated 0.1 M NaCI

B & W Buffer: A suggested 2 x concentrated Binding & Washing Buffer is as follows;

10 mM Tris-HCI (pH 7.5)

1 mM EDTA 2.0 M NaCI

All reagents used should be analytical grade.

STORAGE AND STABILITY

If stored unopened at 2-8°C upon delivery.

NOTE:

The Streptavidin particles must be maintained in liquid during storage and all handling steps. Drying will result in reduced performance. Do not freeze the product.

PRECAUTIONS

Precautions should be taken to prevent microbial contamination of the product. Material Safety Data Sheet is available upon request.