Streptavidin Magnetic Particles Uses and Protocols

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. It has been used both in immune assays and genomic assays for target detection.

PRINCIPLE

Streptavidin bead surfaces are designed as a matrix for simple and efficient methods such as:

• Protein coated beads for the isolation of biotinylated compounds such as proteins, immunoglobulins, sugars, lectins or DNA/RNA and microRNA.

• Magnetic streptavidin coated beads can be used as substrates for both immune assays and genomic assays.

• Small fluorescently labeled streptavidin particles can be used as probes for detection.

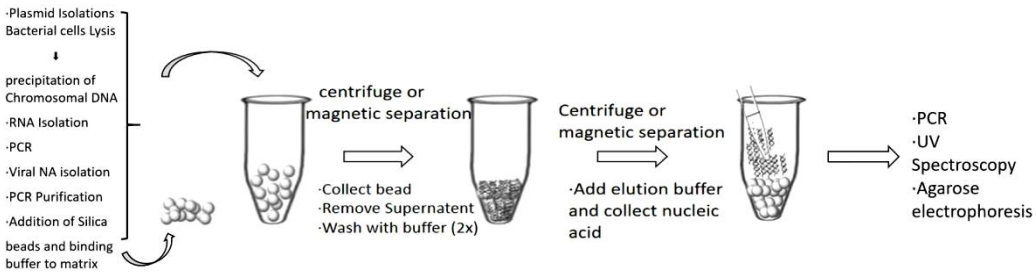

Fig. 1 Capture of Biotinylated Oligos

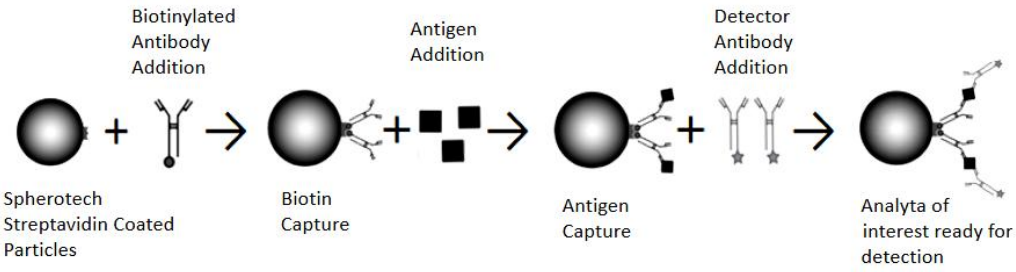

Fig. 2 Sandwich assay and Capture of Biotinylated Antibodies

INSTRUCTIONS FOR USE

Preparations of 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 .

4. Remove the supernatant by aspiration with a pipette. Avoid touching the particle pellet with the pipette tip.

5. Add the recommended buffer along. Use the same volume as in step 2 above and resuspend gently (do not vortex the beads).

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 Streptavidin Particles for RNA anipulations.

NOTE: Streptavidin are not supplied in RNase free solution.

1. Add DEPC to a final concentration of 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 particles twice with the same volume of Solution A for several minutes.

5. Wash the particles once with the same volume of Solution B.

6. Resuspend the particles in Solution B.

BIOTINYLATION PROCEDURES

Biotinylated nucleic acids, proteins and peptides easily bind to Streptavidin Magnetic Particles for isolation experiments. Following the binding procedure, the biotinylated product is maneuvered easily due to the unique magnetic properties of the magnetic particles. The magnetic properties of the particles when placed in a magnetic field simplify handling during experimental procedures such as changing of buffers.

The binding of biotin/streptavidin linking system (KD = 10^{-15} M) results in the strongest non-covalent interaction for the isolation.

NOTES:

- All biotin reagents should contain a spacer arm, at least 6 C-atoms in length, to reduce steric hindrance.
- Free biotin in your sample will reduce the binding capacity of the Streptavidin particles. A disposable column will remove unbound biotin from your sample.
- Biotinylated oligonucleotides should be purified by reverse phase HPLC 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 oligonucleotide synthesis companies.

b) 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 will lead to biotinylate all the primary amine groups of the olignonucleotides. It is recommended to directly incorporate biotin either 5'-end or 3'-end of the oligo during DNA synthesis.

2. Purification of biotinylated primers.

a) It is of great importance that the biotinylated oligonucleotide is pure from unbound biotin, preferably by 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) HPLC purification of biotinylated oligonucleotides is necessary to load full length biotinylated oligonucleotides on the streptavidin coated beads. NAP column purified oligonucleotides effect the loading process.

3. Biotinylation of already synthesised oligonucleotides.

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

4. Biotinylation of larger DNA fragments.

a) It is recommendable to directly incorporate biotin at the 5'-end of the oligo during DNA synthesis using biotin phosphoramidite. The use of a) End-labeling using PCR with a biotinylated primer.

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 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.

5. 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.

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 special pH. At pH 9.5 or greater, iminobiotin will bind tightly to Streptavidin particles. The released iminobiotin can be re-immobilised onto Streptavidin particles.

6. Biotinylation of amino-modified DNA.

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

7. 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 randomly. As with DNA, the photoactivated forms of biotin randomly incorporate into the RNA fragment with UV light.

8. 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 excess of the biotinylation reagent Biotin-X-NHS Ester (MW = 454.5) in DMSO and add this solution to the antibody solution.

3. Add the required amount of NaHCO3 stock solution. Check pH and adjust pH if necessary.

4. Incubate overnight at 4°C.

5. Filter on a gel.

6. Calculate the final concentration of antibodies and store at 4°C. A final concentration of BSA should be added to the biotinylated antibody for stabilization.

7. Number of biotins per antibody can be quantitated using commercially available kit.

9. 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

1. Nucleic acids

1. Wash the 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 concentration suitable for the application of choice.

4. To immobilise, add excess amount of the biotinylated DNA/RNA. 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 20 minutes. DNA fragments up to 1 kb require 30 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, using centrifugation.

8. Resuspend to the desired concentration. The binding is now complete and the beads that are attached with DNA/RNA fragment can be resuspended in a buffer with low salt concentration, suitable for downstream applications.

2. Antibodies.

1. Calculate the amount of biotinylated antibodies needed for immobilization.

2. Mix biotinylated antibody with streptavidin beads and incubate at room temperature with gentle rotation of the tube.

3. Separate theparticles now coated with biotinylated antibodies using centrifugation.

4. Wash 4-5 times in PBS/BSA.

5. Resuspend to the desired concentration.

6. Store the antibody coated beads at 4℃ and do not keep at room temperature.

3. PEG-Biotins

PEG-biotins of different sizes can be immobilized on thestreptavidin beads for different applications following same protocols from DNA immobilization.

BINDING CAPACITY OF DNA TOSTREPTAVIDIN BEADS

The binding capacity ofStreptavidin particles is fragment length dependent. Quantitative assays of labelled PCR product binding to beads show the effect of the length of the PCR product on binding. Shorter oligonucleotides bind fast and more amount on the surface.

Reduced binding capacity for large DNA fragments may be due to secondary structures and cross binding between the oligonucleotides. The salt concentrations influence the efficiency of the binding of biotinylated nucleic acids toStreptavidin particles. Optimal binding conditions for biotinylated DNA fragments ontoStreptavidin particles:

Free biotin and free biotinylated primers (not used during a PCR amplification) bind to the beads much more rapidly than longer PCR amplified products, it is most important to ensure that the solution

containing the PCR product does not contain an excess of unreacted biotinylated primer.

To prevent an excess of free biotinylated primer, one may perform PCR with optimal concentrations of the biotinylated primer and remove the free biotinylated primer using purification methods like HPLC or a purification kit.

NOTE: 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.

One milligram of 4µm Streptavidin particles typically binds:

- Free biotin. Biotinylated oligonucleotide (single stranded).
- Biotinylated antibody depends on the no of biotins per antibody
- a 2-4 kb double stranded DNA fragment.

Buffers and Solutions

Solution A: DEPC-treated 0.1 M Nao, DEPC-treated 0.05 M Nalco

Solution B: DEPC-treated 0.1 M NaCl

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

10 mm Tris-HCl (pH 7.5)

1 mm EDTA

2.0 M NaCl

All reagents used should be analytical grade.

STORAGE AND STABILITY

If stored unopened at 2-8°C upon delivery. Precautions should be taken to prevent microbial contamination of the product.

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

Safety Data Sheet is available upon request.