

Q & A Technically Speaking

Streptavidin MagneSphere® Paramagnetic Particles

Magnetic separation of biomolecules is a rapid, safe alternative to more traditional methods of bioseparation. Promega's Streptavidin MagneSphere® Paramagnetic Particles (SA-PMPs) may be used for the magnetic separation of a variety of molecules by utilizing the strong and specific affinity of biotin for streptavidin.

Q What is meant by 'paramagnetic'?

A paramagnetic (or superparamagnetic) substance is one that exhibits a dipole moment when in a magnetic field but loses the dipole when the field is removed. Simply put, a paramagnetic substance can be attracted by a magnet, but paramagnetic substances essentially do not have a magnetic memory.

Q What are the physical properties of the SA-PMPs?

The SA-PMPs are superparamagnetic, irregular-shaped particles that have a high affinity for biotin. [Table 1](#) lists the physical properties of the SA-PMPs.

Table 1. Properties of Streptavidin MagneSphere® Paramagnetic Particles.	
Property	Streptavidin MagneSphere® Paramagnetic Particles
Composition	Silicized iron oxide.
Shape	Irregular-shaped.
Magnetization	Superparamagnetic (25-35 electromagnetic units/gram measure at 1,000 gauss) with no magnetic memory.
Particle Size	0.5-1.5µm in diameter.
Surface Area	100-150m ² /mg.
Density	2.5g/ml.
Number of Particles	~5 x 10 ⁸ particles/mg.
Concentration	1.0mg/ml.
Storage Buffer	PBS buffer containing 1mg/ml BSA and 0.02% sodium azide.
Structure of Streptavidin	Tetrameric.
Binding Capacity	Nucleic Acid: 0.75-1.25nmol of biotinylated oligo(dT) per milligram particles. Protein: >=70µg of biotinylated IgG per milligram particles.

Q What are some applications of the SA-PMPs?

SA-PMPs can be used in any application where a quality streptavidin-coated paramagnetic particle is required. The SA-PMPs were developed for use in mRNA purification with the PolyATtract® mRNA Isolation Systems (1-4), purification of M13 phage DNA (5) and purification of biotinylated antibodies (3). The SA-PMPs may also be used to capture biotinylated DNA for applications such as: *in*

in vitro transcription, generation of labeled RNA probes and RNA templates (for *in vitro* mutagenesis and *in vitro* translation, 6), study of RNA polymerase II transcription in nuclear extracts (7) and study of *E. coli* RNA polymerase (8). The SA-PMPs have been used for the amplification of 5'- and 3'-ends of cDNAs captured with sequence-specific, biotinylated oligonucleotides (9,10) and to capture DNA for the study of DNA-protein interactions (11,13). Paramagnetic particles have also been used in the construction of subtractive cDNA libraries from limited amounts of mRNA (14,15). Promega's SA-PMPs have been used in a rapid mutation detection procedure to immobilize DNA during chemical cleavage (16).

Q How should the SA-PMPs be stored?

Store the particles at 4°C. **Do not freeze the SA-PMPs or they will clump and be unusable.** Do not let the SA-PMPs dry out.

Q How should the SA-PMPs be prepared before use?

Prior to use, the SA-PMPs should be washed three times with an equal volume of 0.5X SSC and used **within 30 minutes** for optimal performance.

Q How can I test that the SA-PMPs are in good condition?

After the particles are resuspended, they should remain in solution for at least 5 minutes. If some of the particles settle out of suspension within 3-5 minutes, they have clumped together and will not perform optimally.

Q What conditions should be avoided when using the SA-PMPs?

The particles are stable over a temperature range of 4-65°C. Temperatures outside this range will cause the particles to clump and destroy the binding efficiency. SA-PMPs are stable over a pH range of 5.0-9.0. A decrease in performance of the SA-PMPs is observed at SDS concentrations >5.0%.

Q Are the SA-PMPs tested for nuclease and protease contamination?

The Streptavidin MagneSphere[®] Paramagnetic Particles are tested for DNase and RNase contamination using [³H]DNA and [³H]RNA templates. Minimum specifications include <=2% ³H release after incubation of SA-PMPs with the labeled template at 37°C for 5 hours. Protease contamination is assayed by incubating the particles with a fluorescently labeled peptide and comparing the release of label with a standard curve prepared using alkaline protease. Minimum specifications are <0.2ng of protease activity per microgram of particles after a 30-minute incubation.

Q What type of magnet should be used with the SA-PMPs?

A MagneSphere[®] Technology Magnetic Separation Stand should be used to collect the SA-PMPs. The magnet contained in this magnetic stand is made from neodymium cobalt, a powerful rare earth magnet. Rare earth magnets have magnetic strengths several orders of magnitude greater than standard household magnets. This magnetic strength is necessary for efficient collection of the small paramagnetic particles.

Q Can a centrifuge be used instead of a magnet to collect the SA-PMPs?

Collecting the SA-PMPs by centrifugation is not recommended if the particles are to be subsequently resuspended. Centrifugation will cause the particles to clump together making them difficult to resuspend. In addition, centrifugation may trap contaminants within the pelleted SA-PMPs. To ensure good recovery and purity of the sample, a magnet must be used.

Q Can the SA-PMPs be reused?

No, they cannot be reused. Washing the particles removes the BSA required for stability of the particles during long-term storage. In addition, conditions required to break the biotin-streptavidin bond will also destroy the binding capacity of the particles. As such, the particles would need to be reused with the bound biotinylated probe intact. Finally, SA-PMP re-use is not recommended due to the risk

of cross-contamination.

Q Is it possible to break the biotin-streptavidin bond?

The biotin-streptavidin bond is extremely strong. Biotin has a very high affinity ($K_d=10^{15}$ M) for streptavidin, and binding cannot be reversed under nondenaturing conditions. Therefore, we do not recommend the use of the SA-PMPs for applications where the biotinylated molecule will need to be separated and recovered from the SA-PMPs. Note that many applications do not require separation of the biotinylated template from the SA-PMPs (6,7).

Q How do I separate nonbiotinylated nucleic acid from the SA-PMPs?

If the nucleic acid has been captured using a short biotinylated oligonucleotide, the nucleic acid may be eluted by resuspending the nucleic acid/SA-PMP complex in water. This method is used in Promega's PolyATtract[®] mRNA Isolation Systems to elute mRNA captured with the Biotinylated Oligo(dT) Probe. For longer nucleic acid hybrids or those with higher melting temperatures, the nonbiotinylated strand can be separated from the biotinylated strand by heating the hybrid at 95°C for 2 minutes. At Promega we found that it is possible to recover the nonbiotinylated DNA strand from a DNA/DNA hybrid using 0.2N NaOH. To isolate the nonbiotinylated DNA strand from the biotinylated DNA/SA-PMP complex, resuspend the captured DNA/SA-PMP complex in 100µl of 0.2N NaOH. Incubate at RT for 5 minutes, and then neutralize by addition of 10µl 5M ammonium acetate. Add 110µl isopropanol and precipitate the DNA at 20°C for 2 hours to overnight. Collect the DNA by centrifugation, wash the pellet with 70% ethanol and resuspend the DNA in water.

Q What type of biotinylated oligonucleotide is recommended for nucleic acid separations?

We do not recommend the use of long nucleic acid molecules as biotinylated probes because the resulting hybrid may possess a melting temperature (T_m) that is too high to allow elution under practical conditions. In addition, it is desirable to include a long spacer arm (>14 carbons) between the oligonucleotide and the biotin moiety to allow accessibility of the biotin to the streptavidin. For example, the Biotinylated Oligo(dT) Probe supplied with the PolyATtract[®] Systems is a 25mer with a 15-carbon spacer arm.

REFERENCES

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