

MagneSil™ Paramagnetic Particles: Novel Magnetics for DNA Purification



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Promega has developed a new magnetics-based nucleic acid purification technology. In principle, MagneSil™ Paramagnetic Particles (a) work like other purification systems, but add the advantages of increased speed, flexibility and binding capacity. This article introduces the basic physical and performance characteristics of the new MagneSil™ Paramagnetic Particles.

INTRODUCTION

To meet the changing nucleic acid purification needs of researchers worldwide, Promega has developed a proprietary purification matrix. MagneSil™ Paramagnetic Particles (PMPs) use the principle of magnetic separation as an alternative to vacuum filtration and centrifugation separation formats. MagneSil™ Particles also offer a number of advantages over conventional systems, as described here.

Magnetic particles can be considered a "mobile solid phase". As such, the binding of nucleic acids can occur in solution, resulting in increased binding kinetics and binding efficiency. Particles can also be completely resuspended during the wash steps of a purification protocol, thus enhancing the removal of contaminating substances and increasing nucleic acid purity. A further advantage of using magnetic particles for bioseparation applications is their utility with variously-sized starting samples. With most commercially available nucleic acid purification systems, the amount of starting material is limited by the fixed size of the product's purification matrix. No specialized plastics are required with magnetic particles, and since the particles are compatible with most routinely used laboratory plasticware, the amount of magnetic solid phase can be scaled with increasing amounts of starting material. Thus, a single system provides increased flexibility with protocols covering small- to large-scale applications.

There are numerous purification applications for MagneSil™ PMPs in the life sciences and in industrial laboratories. The particles are an integral part of the new Wizard® PureFection Plasmid DNA Purification System (see page 34 for a complete description of this new system). This article highlights the many features of the MagneSil™ PMPs and describes its advantages for the purification of DNA plasmids. (*Promega Notes* 68 featured the application of this technology, the Wizard® PureFection System, for the preparation of high quality, transfection-grade DNA.)

PHYSICAL CHARACTERISTICS OF MAGNESIL™ PARAMAGNETIC PARTICLES

Figure 1 is a scanning electron micrograph of MagneSil™ PMPs. The particles consist of a nearly 1:1 ratio of silicon dioxide (SiO₂):magnetite. The silica chemistry of MagneSil™ Particles is similar to that of the Wizard® Systems, allowing DNA to be bound under like conditions. The high magnetite concentration makes the particles extremely responsive in a magnetic field. The particles are 'paramagnetic' in nature. That is, they are attracted to a magnet when placed in a magnetic field but retain no magnetic "memory" and therefore will not remain magnetized when removed from the magnetic field. This characteristic prevents clumping and allows for easy dispersion of the particles.

MagneSil™ Particles have an average diameter of 5.0-8.5µm and a pore size >500 angstroms. This combination of particle size and macroporous structure results in increased surface area and enhanced binding capacity over similar materials. A unique manufacturing process results in complete encapsulation of the magnetite by SiO₂, which eliminates the possibility of leaching and nonspecific binding to the iron of magnetite.

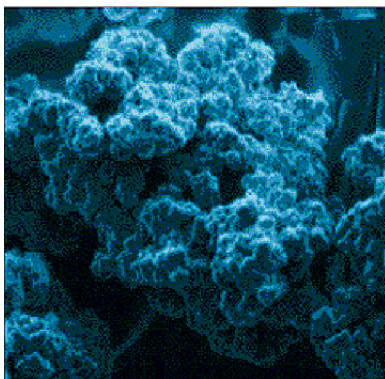


Figure 1. Scanning electron micrograph of MagneSil™ Paramagnetic Particles.

MAGNETIC RESPONSE CHARACTERISTICS

Magnetic response of MagneSil™ PMPs is a concentration-dependent phenomenon (Figure 2). Greater than 90% of the particles are cleared from solution in fewer than 15 seconds at concentrations above 0.5mg/ml. However, even at concentrations as low as 0.01mg/ml, 90% of the particles can be removed from solution in fewer than 45 seconds.

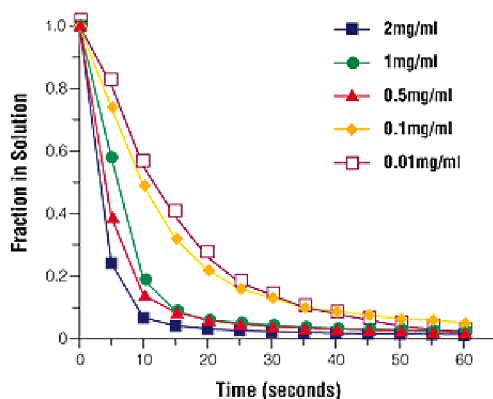


Figure 2. Magnetic response of MagneSil™ Paramagnetic Particles. Magnetic response was measured by placing a mixture of particles and water in a spectrophotometer cuvette holder, which had been modified by attaching a neodymium (S36 grade) magnet on its outside wall. Magnetic response was measured by monitoring the solution at 600nm over time.

This phenomenon is the result of how the particles respond when placed in a magnetic field. At first, a few particles magnetize and self-attract to form a critical particle mass that then moves toward the magnet. At the required particle concentration for most applications, efficient removal of particles is accomplished in under 30 seconds. Faster response times can be achieved by using magnets with enhanced field strengths. Promega's MagneSil™ magnetic stands incorporate S36 grade neodymium rare earth magnets to ensure the highest possible level of performance.

INCREASED BINDING OF NUCLEIC ACIDS

MagneSil™ Particles were engineered for high binding capacity of nucleic acids. The macroporous structure is critical to this feature. Figure 3 shows the effects of particle structure on the binding and recovery of DNA fragments. Lambda DNA/*Hind* III (125-23,000bp) and phiX174/*Hae* III Marker fragments (62-1,306bp) were titrated into fixed concentrations of porous and nonporous particles. Both particle types were of similar average diameter but differed in total surface area. The macroporous particles have a surface area of 27m²/g as compared to 8m²/g for the nonporous materials. As evident in Figure 3, the macroporous particles resulted in increased recovery of DNA over the nonporous materials. This recovery was also inversely related to DNA size with much higher recovery of the smaller phiX174/*Hae* III Marker fragments. For small fragments, final recovery was primarily a function of binding, as these fragments were efficiently eluted in water at room temperature. However, the recovery of lambda fragments was a function of both binding capacity and elution efficiency. The larger fragments have a reduced binding capacity on a weight basis and reduced elution efficiency at room temperature. Uncut lambda DNA of 49,000bp had the lowest recovery of all DNA tested (data not shown).

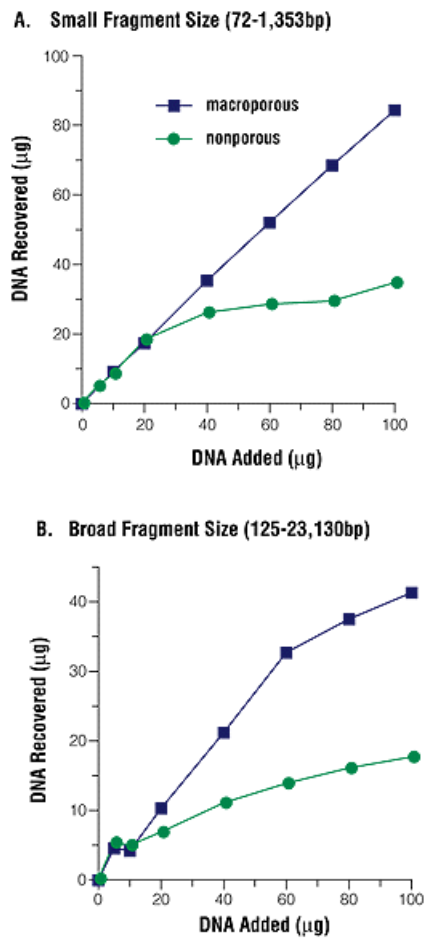


Figure 3. Recovery of Lambda DNA/*Hind* III and phiX174/*Hae* III Marker fragments from macroporous and nonporous MagneSil™ Particles. **Panel A:** PhiX174/*Hae* III Marker fragments. **Panel B:** Lambda DNA/*Hind* III Marker fragments. Increasing amounts of purified DNA were titrated into a fixed concentration of magnetic particles in 4M guanidine HCl at pH 4.5. Following a brief incubation the particles were washed three times with 1ml of Wizard® Plus SV Wash Solution, air dried and bound DNA was eluted in deionized water. Eluted DNA was quantitated by absorbance at 260nm.

A similar set of experiments was performed with bacterial cell lysates (Figure 4). In this experiment, increasing amounts of MagneSil™ PMPs were titrated into cleared bacterial cell lysates prepared from 10ml cultures of DH5alpha® bacteria containing the high copy number pGL3-Control Vector^(b,c) (Cat.# E1741).

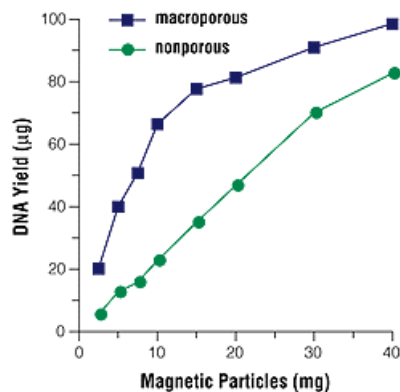


Figure 4. Recovery of plasmid DNA from bacterial cell lysates with macroporous and nonporous magnetic particles. Increasing amounts of MagneSil™ PMPs were added to 10ml of bacterial cultures. Yield of plasmid DNA was determined by absorbance at 260nm.

The results of this experiment are similar to those illustrated in Figure 3. The macroporous particles resulted in the isolation of 80µg of

plasmid DNA for 20mg of particles added to the lysate. In contrast, 40mg of the nonporous particles were required to isolate an equivalent amount of plasmid from a 10ml culture.

SUMMARY

MagneSil™ PMPs represent a new nucleic acid binding matrix with optimized structure for efficient binding of DNA. The SiO₂-encapsulated magnetite composition and macroporous structure combine to exhibit superior performance in plasmid DNA separation. In addition, the MagneSil™ PMPs have been incorporated into a new transfection-grade plasmid purification system, the Wizard® PureFection Plasmid DNA Purification System that is available for midi- and maxiprep isolations. MagneSil™ PMPs enable the Wizard® PureFection System to offer enhanced yields, low levels of endotoxin and proven application performance. These product characteristics result in an excellent value in a plasmid purification system.

Ordering Information

Product	Size	Cat.#
Wizard® PureFection Plasmid DNA Purification System	2 maxipreps/10 midipreps	A2150
	5 maxipreps/25 midipreps	A2160*
	25 maxipreps/125 midipreps	A2170*
MagneSil™ Magnetic Separation Unit		A2231

*The Magnetic Separation Unit is not included with Cat.# A2160 and A2170 and must be purchased separately.

Related Products

Product	Size	Cat.#
pGL3-Control Vector	20µg	E1741
Lambda DNA/Hind III Markers	100µg	G1711
PhiX174/Hae III Markers	50µg	G1761

^(a)Patent Pending.

^(b)U.S. Pat. No. 5,670,356 has been issued to Promega Corporation for a modified luciferase technology.

^(c)The method of recombinant expression of Coleoptera luciferase is covered by U.S. Patent Nos. 5,583,024, 5,674,713 and 5,700,673.

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