

Thermo Scientific Seradyn Sera-Mag[®] Magnetic Oligo(dT) Microparticles

Introduction

The Sera-Mag[®] Magnetic Oligo(dT) microparticles (MG-OL) can be used for a variety of applications:

- mRNA isolation
- cDNA library construction
- Subtractive hybridization
- Affinity purification
- RT-PCR applications
- Primer extension
- cDNA Microarrays

Sera-Mag are nominal 1 micron, super-paramagnetic particles of uniform size with covalently bound oligo(dT)₁₄. Sera-Mag combines the advantages of high surface area, high affinity and high specific activity.

These particles are colloiddally stable in the absence of a magnetic field, however the particles can be separated rapidly and completely from suspension when a magnetic field is applied.

Binding of polyadenylated RNA (poly-A⁺ RNA) to the covalently bound oligo(dT) groups on the surface is easily accomplished using standard hybridization conditions. Other RNA species (rRNA and tRNA) do not contain poly A⁺ and therefore will not bind to the oligo(dT) beads. The isolated mRNA can be used directly in cloning and expression analysis applications.

In addition, the oligo(dT) beads can be used a universal base particle on which to attach your unique oligo sequence. By synthesizing a poly-A tail on your special oligo, you can easily bind this to the dT surface of the particles to create a uniquely coated magnetic particle.

SERA-MAG

Features & Benefits

| Features | Benefits |
|---|--|
| • Covalently bound oligo(dT) ₁₄ | Prevents leaching of oligo(dT) from the particle |
| • Very high surface oligo(dT) concentration | Highest poly A+ binding capacity available. 1 mL of Sera-Mag can bind up to 12 µg of mRNA from cell or tissue depending on expression levels |
| • Surface bound mRNA can be used directly for a variety of molecular biology applications | Versatility of application like cDNA library construction, hybridizations, extractions, purifications and others |
| • Proprietary surface characteristics | Low nonspecific binding of DNA and other proteins, improved isolation efficiency |
| • Encapsulated | No exposed iron, will not interfere with downstream enzymatic applications |
| • Tight size distribution | Even particle separation, good lot to lot reproducibility |
| • Excellent colloidal stability | Monodisperse particle suspension, slow settling rate in absence of magnetic field |
| • Can be sonicated | Versatility in processing |
| • Stable in most buffer systems, pH 5 to 10 | Versatility in performing most applications |
| • Stable in detergents | Compatible with most commonly used detergents (Tween 20, LLS, SDS, etc.) |
| • No residual magnetism after removal of magnetic field | No particle carry over, improved precision |

Product Description

Sera-Mag Magnetic Oligo(dT) particles (MG-OL) are uniform core/shell magnetic microparticles approximately 1 μm in diameter with a magnetite content of approximately 40%. The particles have a rather rough texture, giving a much larger surface area for hybridization than would be available with a smooth surface. Sera-Mag particles are manufactured by using our proprietary manufacturing processes to insure maximum quality and reliability. These particles are designed for various molecular biology applications. These particles feature:

- Covalently bound oligo(dT)₁₄
- Nominal particle diameter of 1 μM
- Packaged at 1% solids
- High oligo(dT)₁₄ surface concentration
- Monodisperse, colloidally stable particle suspensions
- Excellent shelf-life stability
- Low non-specific binding of DNA and protein
- High poly A⁺ binding capacity
- Supplied as a 1% suspension (10 mg/mL)
- Packaged with 0.05% sodium azide preservative
- No surfactants are present
- Nuclease free

Sera-Mag Oligo(dT) Technical Data

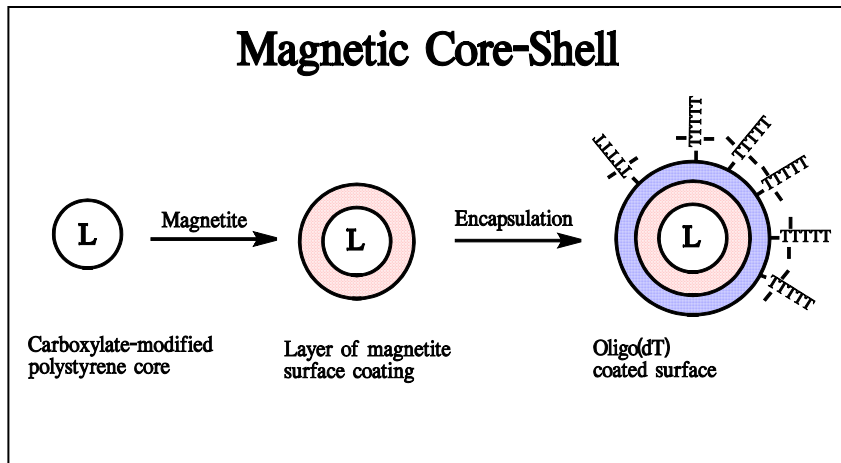
| Nominal Dia. | Size Dist. | Magnetite Content | Density (g/ml) | Mag. Sat. EMU/g | (dA) ₃₀ Binding (pmole/mg) |
|-----------------|------------|-------------------|----------------|-----------------|---------------------------------------|
| 1 μM | $\pm 7\%$ | 40% | 1.5 | 25 | >300 |

These are nominal values. A certificate of analysis is enclosed with each order that includes specific lot analysis. Pasteurization, and autoclaving are not recommended. (dA)₃₀ binding capacity is measured by a luminescent acridinium assay. The (dA)₃₀ binding capacity approximates actual mRNA ligand binding results.

EMU/g is a measure of magnetic responsiveness. EMU/g effects the migration speed to a magnet. The nominal value of 25 EMU/g for Sera-Mag is significantly greater than competitive magnetic particles.

Sera-Mag have a shelf-life of 2 years from date of manufacture. Recommended storage of Sera-Mag is 2°C to 8°C.

Sera-Mag Magnetic Oligo(dT) Production Process



Sera-Mag Production Process

Carboxylate-modified polystyrene core particles are coated with magnetite and encapsulated with a proprietary polymer coating. Oligo(dT)₁₄ is then covalently bound to the surface.

Magnetic Response Time

In the absence of any magnetic field, no significant sedimentation takes place in an hour. It should be noted that suspension conditions will have a great effect on the speed of magnetic response. In general, conditions that increase the colloidal stability will decrease the magnetic response rate. For example, increasing pH or raising the buffer viscosity will slow the movement of magnetic particles toward a magnet. Particles may be repeatedly separated by a magnetic field and redispersed with no residual magnetism.

General Usage Conditions

We are a manufacturer of exquisite microparticles. Exact mRNA isolation applications information has not been fully developed for these particles. Use these general recommendations when working with Sera-Mag Oligo(dT) microparticles and then modify to your specific application conditions.

- When possible, perform mRNA extractions from isolated total RNA
- As a starting point, use 10 µg of (dT) beads per mRNA extraction
- Hybridization takes place within 5 to 15 minutes
- Hybridize at temperatures between room temperature up to 40 °C
- We recommend using lithium lauryl sulfate (LLS), SDS or N-lauroyl sarcosine to inactivate any endogenous RNase
- Include surfactants with hybridization buffer to denature any protein
- We recommend buffer conditions >0.5 molar using monovalent cations such as sodium, lithium, potassium or ammonium.
- Use pH >5.5 and <9.0
- Sodium azide may be used as a preservative (0.02% to 0.1%)
- Stable in 95 °C PCR cycling temperatures
- Stable in guanidium thiocyanate extraction buffers
- Stable in DMF and DMSO up to ~20%

Conditions to Avoid

Although Sera-Mag Magnetic Oligo(dT) particles are very stable, some conditions should be avoided:

- Avoid freezing the particles
- Avoid temperatures above 95° C
- Avoid pH <5 and >12
- Avoid conditions where RNase contamination may occur

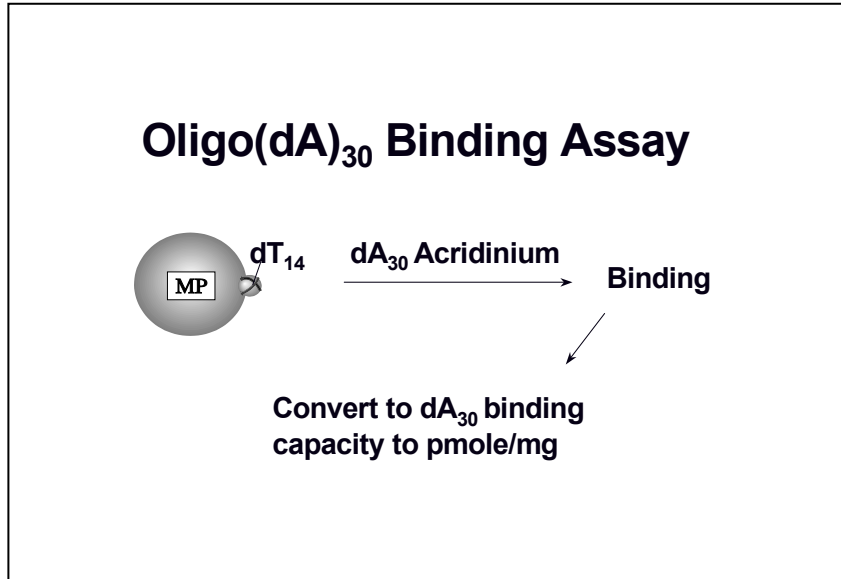
Usage Notes

Given the huge excess of rRNA from cells, even with low efficiency of non-specific capture, one will still get some amount of rRNA bound. Typically, a polyA mRNA prep will have about 1-5% contamination with rRNA. A second cycle of binding/capture will clean it considerably. This artifact will usually be less on dT14 than dT25.

The best way to increase stringency is increasing the temperature of binding, which these particles can take easily. One needs to strike a salt/temperature balance after experimentation.

RNase - The dT particles are typically used in lithium lauryl sulfate. Most RNA extraction procedures using beads use either LLS, SDS, or N-lauroyl sarcosine. Any of these would render endogenous RNase inactive. The base magnetic particles, oligo sequence, and coupling procedures are all the result of organic syntheses (no enzymes) and there are no blocking agents such as BSA used during any of the steps. Therefore, there is no source of potential RNase.

(dA)₃₀ Binding Capacity

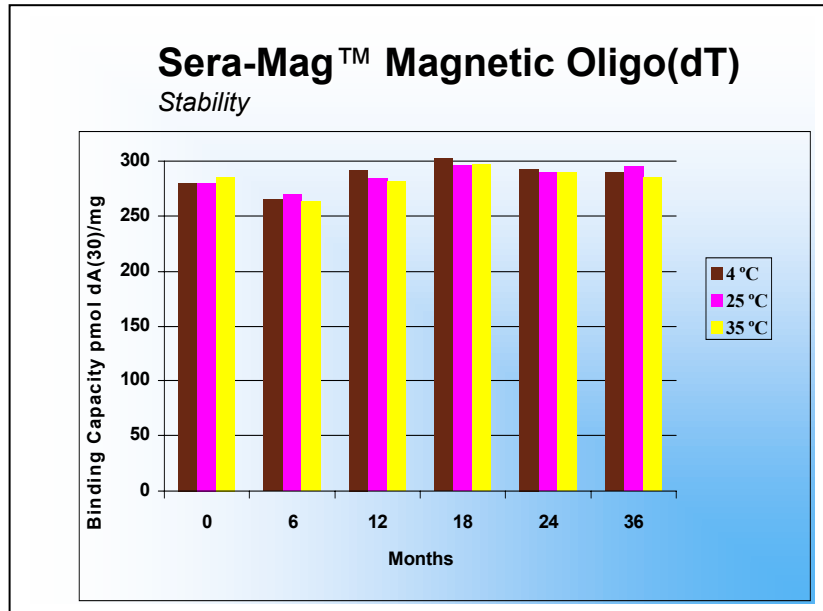


Sera-Mag poly A+ Binding Capacity

Determining the amount of oligo(dT)₁₄ bound to the particles and the capacity to bind poly A+ are important analytical steps. Actual mRNA binding capacity in clinical samples is dependent upon the mRNA length, poly A+ tail, suspension conditions, presence of RNase, pH, sample type (tissue, cell, serum, urine, etc.) and expression level of the mRNA.

This (dA)₃₀ analytical method allows for a consistent measurement method to determine the functional binding reproducibility from lot to lot. The analytical method binds an acridinium labeled (dA)₃₀ probe to the oligo(dT)₁₄ particles and measures a chemiluminescent change proportional to the quantity of the probe bound to the surface of the particles.

(dT)₁₄ Stability Data

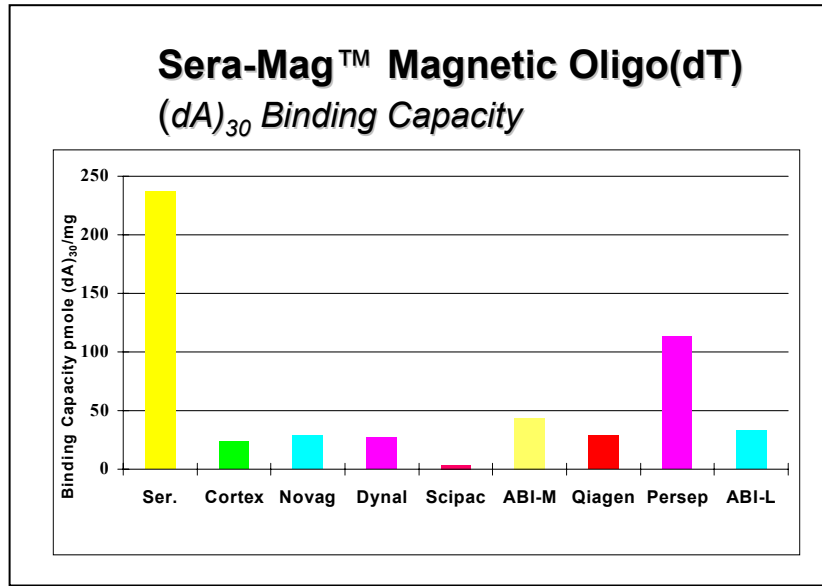


Sera-Mag Magnetic Oligo(dT)₁₄ Stability

A manufactured lot of magnetic oligo(dT)₁₄ particles was incubated at 4° C, 25° C and 35° C to examine the stability of the particles as a function of time and heat stress. There is no significant difference between the data at any temperature, and little variability through the testing period. The average is 5.8 BCU/ug (which equates to a (dA)₃₀ binding capacity of approximately 290 pmol/mg) with a CV% of 5%. This method compares well with actual mRNA isolation applications.

Sera-Mag have a shelf-life of 3 years based on real time studies. Based on accelerated stability testing using the Arrhenius rate equation, the Sera-Mag Oligo(dT) have a shelf life of 4 years.

(dA)₃₀ Binding Comparisons



Sera-Mag (dA)₃₀ Binding Comparisons

We applied the (dA)₃₀ binding assay to a comparison among several commercial sources of magnetic oligo particles. Among the particles tested, Seradyn had the highest (dA)₃₀ binding capacity. This demonstrates the effectiveness of Sera-Mag's unique surface for high capacity binding.

This comparison was run with the new improved direct (dA)₃₀ binding assay. The prior indirect method only gave an estimate of (dA)₃₀ binding capacity, whereas the new method is a direct measurement.

References

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BCA Protein Assay Reagent Bulletin, Pierce Chemical Co., Rockford, IL.

K. Weichelman, T. Braun and J. Fitzpatrick, *Anal. Biochem.* 175 (1998) 231-237.

Korsnes, L. and E. Hornes (1990) Magnetic DNA Hybridization Properties of Oligonucleotide Probes Attached to Superparamagnetic Beads and Their Use in the Isolation of Poly(A) mRNA from Eukaryotic Cells. *GATA* 7(6): 145-150.

**Ordering
Sera-Mag®
Magnetic
Oligo(dT)
Microparticles**

Sera-Mag Magnetic Oligo(dT) Microparticles, 1 µM
(Nominal diameter - 1% solids concentration, 0.05% sodium azide)

| Sera-Mag Magnetic Oligo(dT) | | Catalog No. |
|-----------------------------|-------|------------------|
| 1 µM | 1 mL | 2815-2103-011150 |
| 1 µM | 5 mL | 2815-2103-010150 |
| 1 µM | 15 mL | 2815-2103-010250 |

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Products**

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