

Sera-Mag® Magnetic Streptavidin Microparticles

Introduction

The Sera-Mag Magnetic Streptavidin microparticles (MG-SA) can be used for a variety of applications:

- Improvement and simplification of ligand binding
- Molecular biology applications
- Affinity purifications

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

These particles are colloidally 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 biotinylated ligands to streptavidin groups on the surface is easily accomplished using standard avidin-biotin technology.

In the past, achieving high activity and stable binding of solid phase ligands has been a major difficulty. Compounds which are difficult to attach to microparticle (MP) surfaces by conventional means may be amenable to biotinylation. Due to the high affinity of the avidin-biotin reaction, binding biotinylated compounds to Sera-Mag may improve specific activity. In such cases, biotinylation may be carried out in either aqueous or organic solvent; then the biotin derivative can be bound to Sera-Mag simply by mixing in appropriate buffer conditions. For example, nucleic acids which adsorb poorly to MP surfaces are readily bound to Sera-Mag after biotinylation.

The use of magnetic microparticles as a solid phase support in immunoassays and molecular biology applications has been well documented. Standard protocols are available to biotinylate a wide range of ligands including proteins, nucleic acids, haptens, peptides, etc. Several sources for biotinylation procedures are readily available i.e. *Avidin-Biotin Chemistry: A Handbook*, Pierce Chemical Co., 1992.

SERA-MAG

Features & Benefits

Features	Benefits
• Dissociation constant (K_d 10^{-15} molar)	Biotinylated compound is >95% irreversibly bound to streptavidin (SA)
• Covalently bound streptavidin	Prevents leaching of streptavidin from the particle
• One-step binding protocol	Simple process, solve difficult coupling problems easily
• Proper surface orientation of biotinylated compound	High activity of surface bound ligands, lower ligand load requirements. Decreased particle quantity required per assay
• Encapsulated, proprietary polymer surface	Low nonspecific interactions, improved assay accuracy
• Can be used in EIA formats with biotin/enzyme detection systems	Versatility of application
• Multiple surface SA concentrations available	Allows for optimization and diversity in your applications development work
• Encapsulated	No exposed iron
• Tight size distribution	Even particle separation, good lot to lot reproducibility, efficient use of biological reagents
• Excellent colloidal stability	Monodisperse particle suspension, slow settling rate in absence of magnetic field
• Can be sonicated	Versatility in processing
• Stable in biological buffer systems, pH 5 to 10	Versatility in reagent preparation, compatible with most commonly used buffers
• Stable in detergents	Versatility in reagent preparation, compatible with most commonly used detergents (Tween 20, Triton X100, SDS, etc.)
• No residual magnetism after removal of magnetic field	No particle carry over, improved assay precision

Product Description

Sera-Mag Streptavidin Magnetic particles (MG-SA) are 1 μm in diameter with a magnetite content of approximately 40%. The particles have a rather rough surface, giving a much larger surface area for coupling than would be available with a smoother surface. Sera-MagTM particles are manufactured by Seradyn using proprietary manufacturing processes to insure maximum quality and reliability. These particles are designed for various applications such as diagnostic assays, biomedical research, cell separation and nucleic acids research. These particles feature:

- Covalently bound streptavidin
- Nominal particle diameter of 1 μM
- Packaged at 1% solids
- Low to high streptavidin surface loadings available
- Monodisperse, colloidally stable particle suspensions
- Excellent shelf-life stability
- Low non-specific binding of serum proteins
- High biotin binding capacity

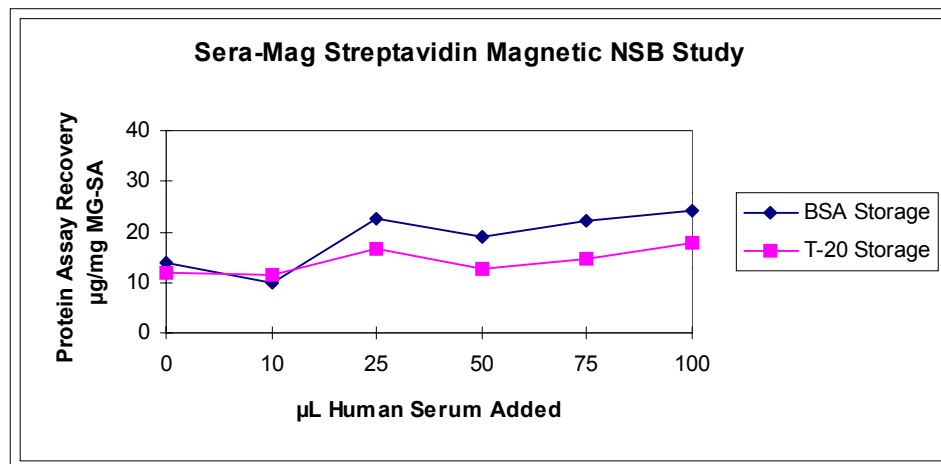


Fig. 1
Non-specific Binding Properties of Sera-Mag Magnetic Streptavidin
Initial surface bound protein level (streptavidin coating) was determined by Seradyn's "Microparticle-Bound Protein Assay". Sera-Mag were prepared with either BSA or Tween-20 based storage buffer solutions. Aliquots of Sera-Mag were then mixed with various concentrations of human serum. After incubation, the surface bound protein level was measured again in an attempt to determine the level of non-specifically bound protein from serum.

The data show that Sera-Mag has very minimal non-specific adsorption. This test was performed with very high levels of added serum proteins. The minimal non-specific adsorption of serum proteins is very important in preventing non-specific interactions during serum-based immunoassays. This can lead to much better assay precision.

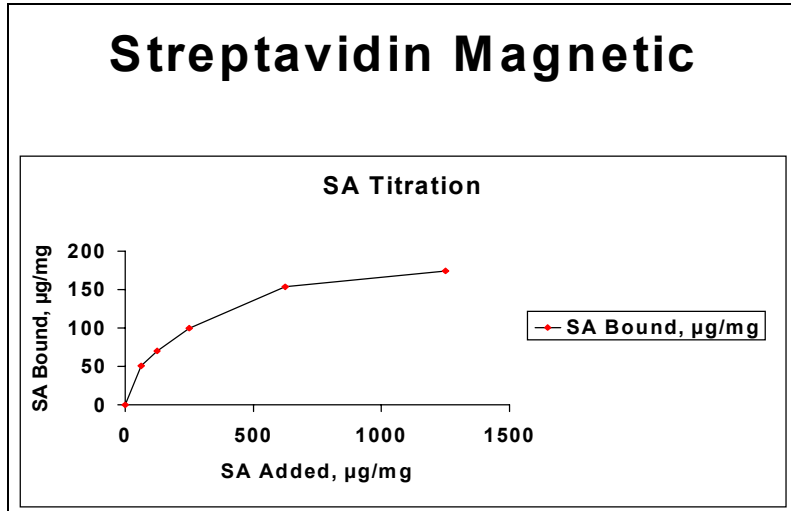


Fig. 2
Multiple Streptavidin Surface Loadings

The base Sera-Mag Magnetic carboxylate-modified particles (MG-CM) covalently bind streptavidin (SA) in proportion to the amount of added SA. Surface streptavidin concentration can be precisely controlled over a wide range. Bound streptavidin is assayed using Seradyn’s “Microparticle-Bound Protein Assay”. Functional activity is measured with the biotin fluorescein assay. The ability to control the surface loading of streptavidin is very important in optimizing your project needs. Sera-Mag Magnetic Streptavidin is available in five streptavidin surface concentrations.

**Sera-Mag
Streptavidin
Technical Data**

Nominal Size Dia.	Size Dist.	Magnetite Content	Density (g/ml)	Mag. Sat. EMU/g	Biotin Binding (pmol/mg)
1 µM	±5%	40%	1.5	25	1000 to 5000

These are nominal values. A certificate of analysis will be enclosed with each order that includes specific lot analysis. Pasteurization, and autoclaving are not recommended. Biotin binding capacity is measured by a biotin fluorescein assay. The binding capacity closely approximates actual biotinylated 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 Magnetic Streptavidin Production Process

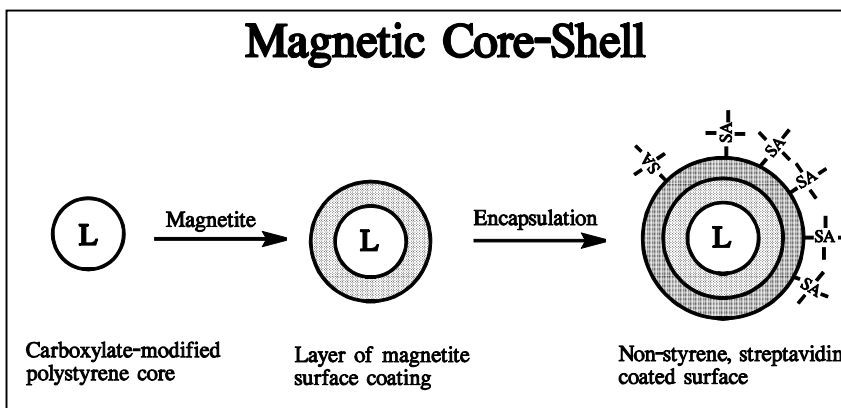


Fig. 3 Sera-Mag Production Process

Carboxylate-modified polystyrene core particles are coated with magnetite and encapsulated with a proprietary polymer coating. Streptavidin 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 solution conditions and the binding of biotinylated compounds will have a great effect on 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.

Potential Applications

- Molecular Biology Formats
- Cell Isolation Formats
- Bio Purification

General Usage Conditions

Use these recommendations when working with Sera-Mag Magnetic Streptavidin Microparticles

- Maintain an excess of biotinylated ligand to prevent crosslinking of

- particles through multiple biotin-SA bonds
- For binding reactions, use buffers with pH >7.0 (phosphate, tris, MOPSO, and similar buffers)
 - Maintain salt concentration of 100-150 mM
 - Mix Sera-Mag and biotinylated ligand for approximately 1 hour at room temperature
 - After binding, wash with buffer by either centrifugation or tangential flow filtration methods
 - Sodium azide may be used as a preservative (0.02% to 0.1%)

Biotin Binding Capacity

Determining the amount of streptavidin bound to the particles and their capacity to bind biotin are important analytical steps. Biotin-binding capacity is usually determined by two methods, biotinylated probes or biotinylated enzymes. The probe method binds a biotinylated probe to the streptavidin and measures a spectral change proportional to the quantity of the probe present. Most of the probes used develop color from the binding or hydrolysis of a chromophoric moiety. HABA (2-hydroxyazobenzene-4'-carboxylic acid) forms a yellow-orange complex with streptavidin.

A more common method uses a biotinylated enzyme such as a peroxidase, phosphatase, or galactosidase. Colorless enzyme substrate is cleaved to form a colored solution whose intensity is directly related to the amount of enzyme present.

Each of these methods has drawbacks. HABA binds weakly to streptavidin, limiting sensitivity and complicating analysis. Enzymes may vary considerably in specific activity depending on pH, buffer, and temperature. This makes standardization difficult and limits precision.

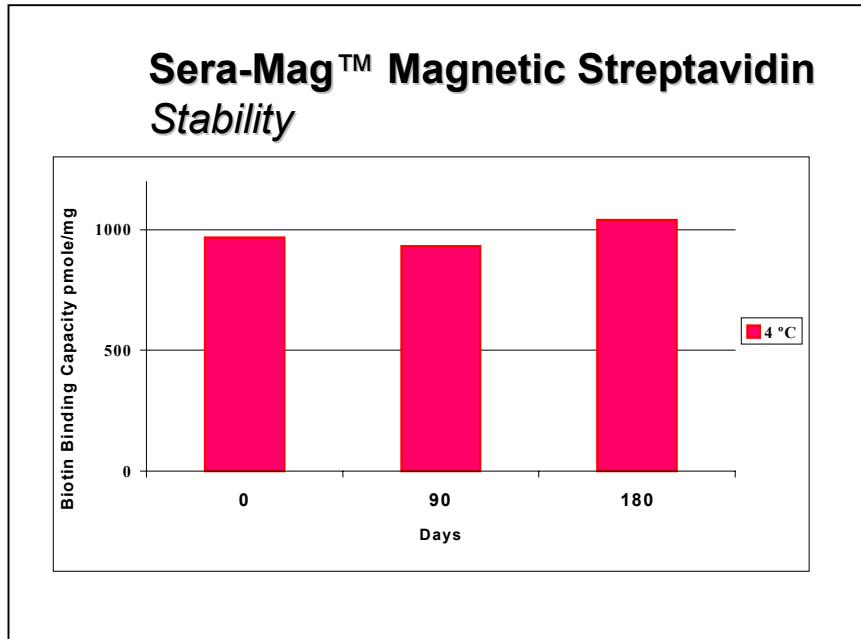


Fig. 4 Sera-Mag Magnetic Streptavidin Stability

Additional support for the reproducibility of the assay can be seen in Figure 4. A manufactured lot of streptavidin magnetic particles was incubated at 4° and 37°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 4° and 37° for any given day, and little variability through the testing period. The average is 2886 pmole/mg with a CV% of 8.8%. This method compares well with other assays.

Biotin-Binding Comparisons

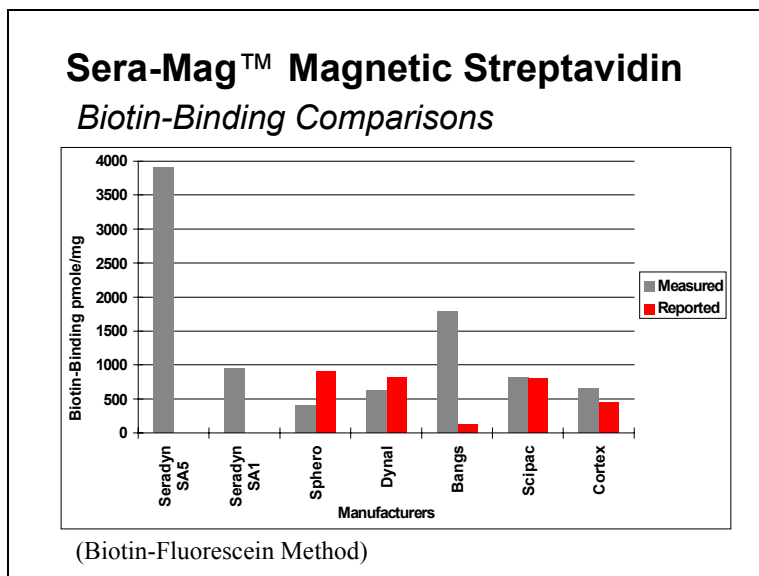


Fig. 5 Biotin-Binding Comparisons

We applied the Biotin Fluorescein method (see p. 11) to a comparison among several sources of magnetic streptavidin particles. Some of the values obtained from this method agreed fairly well with the values reported in the package inserts accompanying the particles. Among the particles tested, Seradyn had the highest biotin-binding capacity. For one particle we obtained a BF value much greater than that reported by the manufacturer (Bangs). The value reported by this manufacturer uses a large biotinylated probe molecule in the determination of their biotin-binding capacity. It is possible that this probe gives lower biotin-binding values due to steric inhibition.

Surface-Bound Streptavidin (BCA)

We have defined a procedure in which the amount of streptavidin bound to the particle is determined with an assay that does not require the particles to be present while the samples are read. The *Microparticle-Bound Protein Assay* [using the bicinchoninic acid (BCA) assay from Pierce, Inc.], satisfies this need. In this assay, Cu^{+2} reacts with oxidizable amino acids to form Cu^{+} which then complexes with BCA to produce a blue to violet color. After the surface proteins have reacted with the copper ions, the particles are removed by filtration or centrifugation and the absorbance of the supernatant measured. See our *Microparticle-Bound Protein Assay* technical notes.

The streptavidin concentration of the particle sample is calculated from a quadratic plot of absorbance as a function of concentration using ten standards ranging from 0 to 1 mg/mL. Particle streptavidin concentration is calculated by dividing the particle sample streptavidin concentration by the particle concentration expressed in mg/mL (1% = 10 mg/mL). Note: The procedure can also be performed on a microscale using a 96-well microplate.

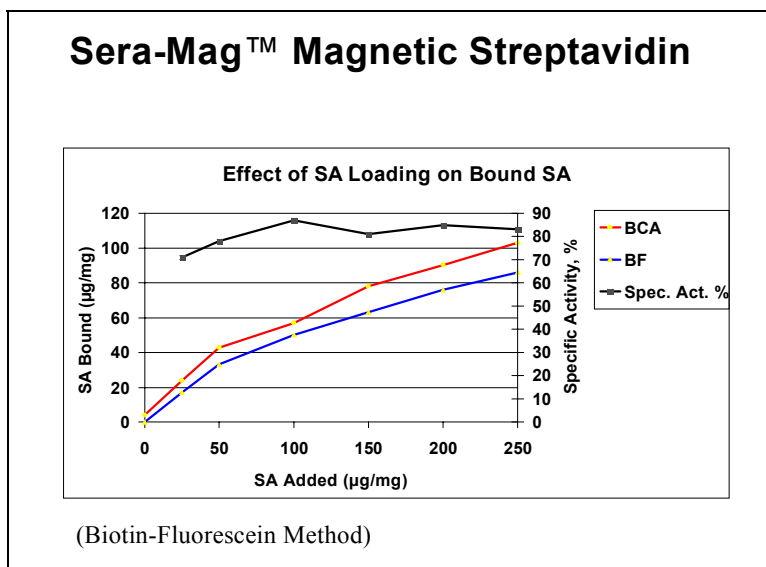


Fig. 6 Sera-Mag Specific Activity

It is useful to compare the values obtained from an assay for the amount of bound streptavidin with the biotin-binding capacity. This permits the measurement of the specific activity of the bound streptavidin on the surface of the microparticle.

Figure 6 shows the specific activity at varied levels of streptavidin applied during coating. As one might expect, the measured biotin-binding capacity on the particles is somewhat lower than the capacity calculated from bound protein. This is probably due to the loss of one or more binding sites per tetramer of streptavidin that is between the surface of the particle and the streptavidin molecule. The specific activity BF/BCA remains above 70% throughout the applied SA range. This corresponds to the loss of approximately 1 biotin-binding site per SA molecule bound.

Biotin-Binding Determination Methods

Table 1
Biotin-Binding Method Comparison
(all measurements given per mg particles)

Lot	BAP Assay		H ³ - Biotin Biotin (pmole)	BF Assay	
	SA (µg)	Biotin (pmole)		SA (µg)	Biotin (pmole)
7ARN	44.9	2721	3000	46.4	2812
7ARP	31.0	1879	2880	43.4	2630
7ARR	40.9	2479	2840	44.7	2709

Table 1 shows data from three assays representing the major biotin-binding determination methods. The three lots of streptavidin magnetic particles were manufactured under identical conditions. The data at the left represent a single run using the BAP enzymatic assay

described earlier. While 7ARN and 7ARR have nearly the same biotin-binding capacity, they are quite different from 7ARP. Data in the center were obtained using a tritiated biotin method, and the data at the right show the results of the BF assay. The BF and tritiated assay methods are in close agreement, but the BAP assay gives an erroneously low result. We attribute this problem with the BAP method to assay imprecision.

Because the BAP assay is so time consuming, performing multiple assays of a given lot is more difficult. Using a fluorescent method such as described in these technical notes makes performing multiple assays easier, thereby giving more reliability to the assigned biotin-binding values.

Biotinylated Alk-Phos Method

The biotinylated alkaline phosphatase (BAP) method is probably used more often because of the ready availability of assays for this enzyme. In our procedure, SA-coated particles are mixed with an excess of BAP. The amount of bound enzyme activity is measured by following the increase in absorbance at 405 nm due to hydrolysis of the substrate, p-nitrophenyl phosphate. The reaction rates are measured and graphed vs. the amount of biotin added (in nanograms) to the reaction. The added biotin at the intersection of the asymptotes from each end of the hyperbolic line is divided by the amount of particles added (mg) to give the biotin-binding capacity.

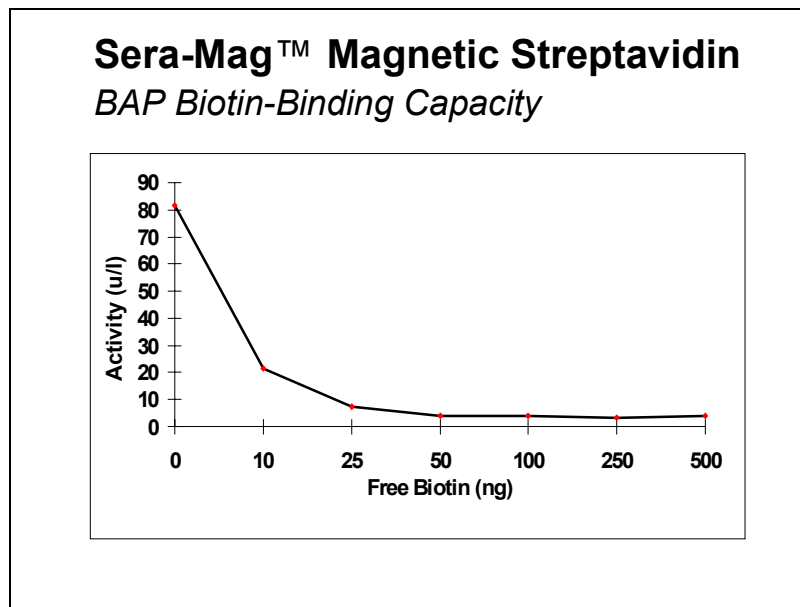


Fig. 7 Biotin Alkaline Phosphatase Biotin-Binding Capacity

In practice, when a typical graph appears like this, with nearly complete inhibition at 25 ng biotin, additional assays using biotin concentrations between the 0 and 25 ng levels should be run so that a more smooth curve can be created. The biotin-binding capacity is taken at the point of 95% inhibition of BAP binding.

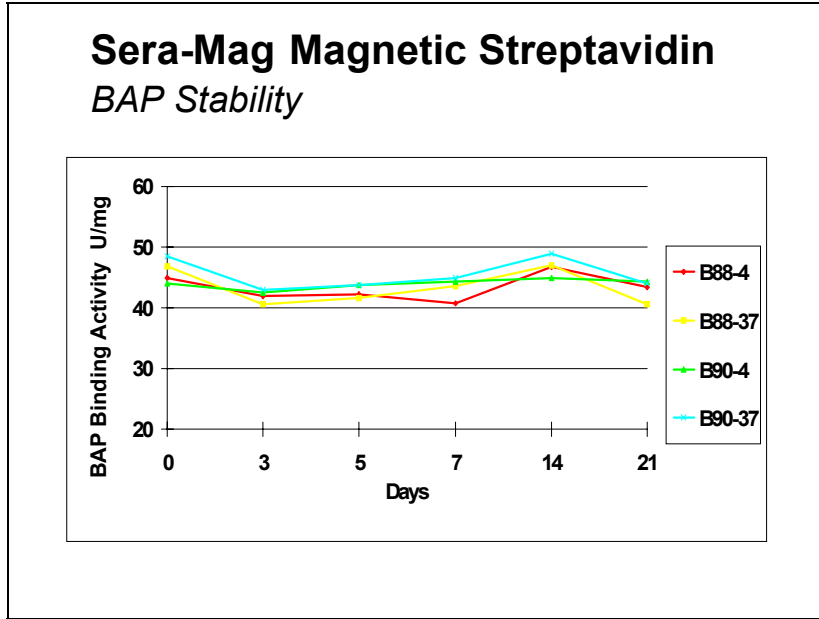


Fig. 8 Biotin Alkaline Phosphatase Stability Data

If performed correctly, the precision and reproducibility of the BAP assay is satisfactory. This is supported by the results of a stability study performed on two different magnetic streptavidin particles.

Table 2: Biotin-Binding Capacity (given per mg particles)

Streptavidin Level	Biotin (pmole)
Low	1810
Medium	2760
High	5700

Typical biotin-binding capacities, expressed in pmole biotin per mg particle, for three bound streptavidin levels are shown here.

Biotin Fluorescein Method

We have developed an easier method using biotinylated fluorescein (BF) to measure the biotin-binding capacity. This method requires much less manipulation which results in improved precision. In this assay, the streptavidin particles are incubated with biotinylated fluorescein (BF) [5(6)-(biotinamidocaproylamido)-pentylthioureidyl-fluorescein]. The particles are centrifuged out and the fluorescence of an aliquot of supernatant is measured. The biotin-binding capacity is calculated from the difference between the fluorescence of the BF in the presence and absence of particles.

$$BF_b, \mu\text{mol/L} = \left(1 - \frac{\text{sample fl.}}{\text{"blank" fl.}}\right) (0.909 \mu\text{mol/L BF})$$

Where “fl” is fluorescence, the working BF solution is 1 $\mu\text{mol/L}$ with a final concentration of 0.909 $\mu\text{mol/L}$ and BF_b is bound BF.

$$\frac{\mu\text{g SA}}{SV, \text{mL}} = (BF_b, \mu\text{mol/L}) \left(\frac{1.1 \text{ mL}}{SV, \text{mL}} \right) \left(\frac{16.5 \mu\text{g SA}}{\text{nmole}} \right)$$

Note: “SV.” is sample volume and 1.1 mL is the assay volume. The biotin-binding calculation assumes that 1 streptavidin subunit of Mr 16.5 g/mol is bound per mole of BF.

$$\frac{\mu\text{g SA}}{\text{mL MP}} = \frac{\left(\frac{\mu\text{g SA}}{\text{mL sample}} \right)}{\left(\frac{\text{mg MP}}{\text{mL sample}} \right)}$$

Note: The denominator is related to the percent solids (1% = 10 mg/ml). “MP” is microparticle.

Biotin-binding capacity (pmole biotin/mg MP):

$$\frac{\text{pmole biotin}}{\text{mg MP}} = \frac{\left(\frac{\mu\text{g SA}}{\text{mg MP}} \right)}{\left(\frac{0.0165 \mu\text{g SA}}{\text{pmole}} \right)}$$

Samples are read on a fluorometer using the appropriate filters for fluorescein analysis. We use a Shimadzu RF1501 fluorometer with an excitation filter of 470 nm and an emission filter of 520 nm. The values are used to determine the amount of active streptavidin subunit bound ($\mu\text{g SA}$ subunit / mg particle) and the biotin-binding capacity (pmole biotin / mg particle) as described above.

**Biotin
Fluorescein
Method**

Table 3
Biotin Fluorescein Results

Sample	Biotin 0 μ M	Biotin 10 μ M
None (Buffer only)	1583	1430
Lot A (Low SA)	1032	1426
Lot B (Medium SA)	342	1393
Lot C (High SA)	133	1410
Base Particles	1465	1589

Table 3 shows the results of the assay for five samples. The first sample contains only particle storage buffer. Three preparations of streptavidin magnetic particles are evaluated for their biotin-binding capacity. The fifth sample is the base particle with no streptavidin bound. Samples were pre-incubated with and without a high level of biotin (10 μ M) before the biotin fluorescein was added. The fluorescence of the sample containing base particle free of streptavidin is about the same as the sample containing only buffer. This is true at both levels of pre-incubated biotin. This demonstrates that the magnetic particles do not interfere with the assay. Preincubation with free biotin (10 μ mol/L) largely inhibits the binding of BF, thus showing that BF binding is specific. Taken together, these data show that BF does not bind nonspecifically to the Sera-Mag magnetic particles.

**Biotin
Fluorescein
Method**

Table 4. Biotin Fluorescein Within-Run Precision

Sample	Buffer	A	B	D
1	1618	1235	650	328
2	1613	1239	643	377
3	1601	1222	582	372
4	1603	1227	593	375
5	1603	1224	597	371
6	1597	1218	587	371
7	1604	1226	646	369
8	1577	1232	611	366
9	1593	1231	599	384
10	1595	1222	602	378
Avg	1600	1228	611	369
St.Dev.	11.2	6.6	25.7	15.3
CV%	0.70	0.53	4.20	4.15

Table 4 shows the within-run precision for the assay using buffer samples and three levels of bound streptavidin. The data are shown in relative fluorescence units. The within-run precision (<5%) is acceptable for all samples.

Table 5. Biotin Fluorescein Between-Run Precision

Day	A	B	D
1	15.9	41.2	51.4
2	14.3	39.2	49.5
3	15.9	40.8	50.2
4	15.4	41.1	51.6
5	15.2	39.9	51.7
6	14.3	40.1	50.3
7	13.9	39.3	49.1
8	16.0	40.7	50.8
Ave:	15.1	40.3	50.6
St. Dev.:	0.84	0.78	0.98
CV%	5.57	1.93	1.94

Table 5 shows the between-run precision. The between-run precision (<6%) is acceptable for all samples.

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**Ordering
Sera-Mag™
Magnetic
Streptavidin
Microparticles**

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

Sera-Mag are supplied in various streptavidin (SA) surface concentrations:

Surface Concentration - Level 1		Catalog No.
1 µM	1 mL	3015-2101-011150
1 µM	5 mL	3015-2101-010150
Surface Concentration - Level 2		
1 µM	1 mL	3015-2102-011150
1 µM	5 mL	3015-2102-010150
Surface Concentration - Level 3		
1 µM	1 mL	3015-2103-011150
1 µM	5 mL	3015-2103-010150
Surface Concentration - Level 4		
1 µM	1 mL	3015-2104-011150
1 µM	5 mL	3015-2104-010150
Surface Concentration - Level 5		
1 µM	1 mL	3015-2105-011150
1 µM	5 mL	3015-2105-010150

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