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Tech. Note #12
MAGNETIC MICROSPHERES

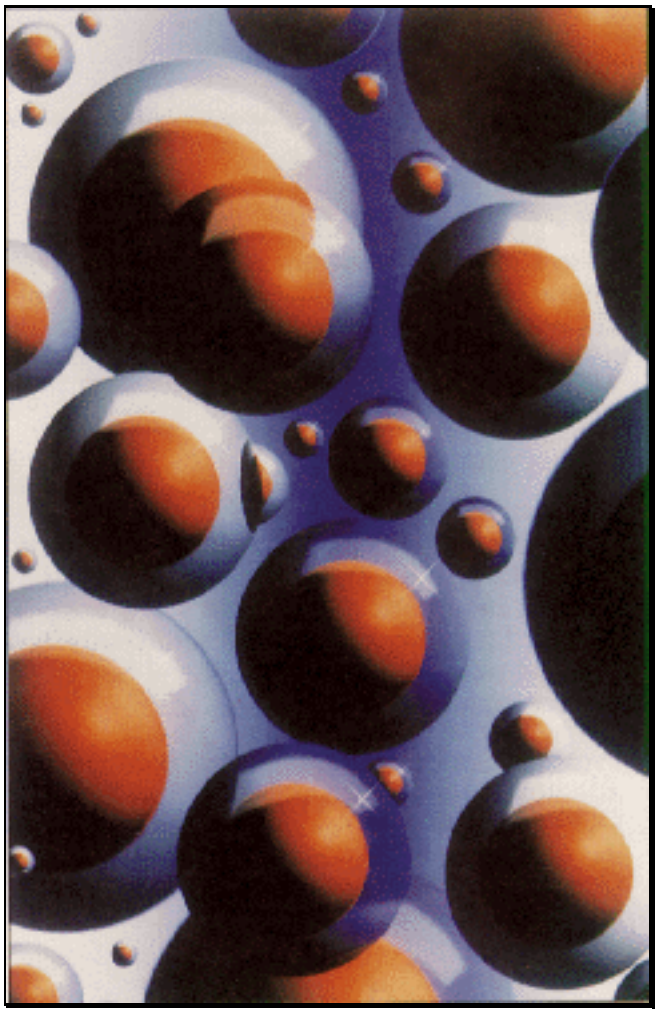
Our estapor® **carboxylate-modified classical** superparamagnetic microspheres are supplied as a brown aqueous dispersion with 10% solids content. The four varieties, containing 12, 20, 40, and 60% magnetite, have surface charges of 25-170 meq of COOH/g. Ferrite crystals of 1–20 nm are dispersed in styrene/divinylbenzene monomers and polymerized by the suspension process, yielding magnetite-laden S/DVB-based microspheres with median diameters of ~0.7–1.3µm. Because the densities are low (~1.1–2.2 g/ml), microspheres remain well dispersed in solution .

Quickly responding to a magnet, the particles are ideal for solid phase assays and have been used for years for radioimmunoassays, ELISA, cell separation, and more recently for chemiluminescent immunoassays and various applications in molecular biology . They have a proven record of ~13 years in commercial use. Lot sizes up to 6 kg (~60 liters at 10% solids) can be produced. These *hybrid* microspheres, allow adsorption *or* covalent linkage of proteins to the carboxylic acid-modified styrene surfaces.

We also offer **amino-modified classical** magnetic microspheres with 40 and 60% magnetite. They facilitate the use of different binding chemistries and are especially useful for *DNA probe and PCR work*.
One can covalently bind to the primary amine groups by the usual methods, *i.e.* via glutar-aldehyde, N-hydroxysuccinimide linkers, or water-soluble carbodiimide.

Our high DVB-content particles permit chemical syntheses in organic solvents.

In response to requests from customers doing *cell depletion work* , where very small particles are a hindrance, a line of **narrow size distribution** materials are now offered. These new materials were specially synthesized to produce a narrower size distribution than the classical microspheres.



Encapsulated SuperparaMagnetic Microspheres
Psychedelic Electron Microscopy
(Artist's Conception)

Standard deviations are shown in the data table following. We presently offer the narrow distribution particles with 12, 40 or 60% magnetite.

Encapsulated microspheres are for customers who need magnetic microspheres with *no iron at the surface*. Exposed iron, or Fe_3O_4, interferes in the functioning of certain enzymes, like polymerase. These new 20, 30, or 40% magnetite microspheres consist of a magnetite-rich core, isolated by a pure polymer shell. These

microspheres, truly designed for PCR, also have a narrower distribution, with superior clearing properties: the 40% magnetite encapsulated particles yield a clear supernatant faster even than the classical 60% magnetite microspheres. Lot sizes are now as large as 1.3 kg of solids. Most lots have the usual COOH modified surface, but several research lots have primary **NH2 surface chemical groups** for alternative coupling.

New surface chemistries recently introduced as research products include **hydrazide** and **chloromethyl** functional groups.

Surface Properties

The surface is largely composed of polystyrene and carboxylic acid (–COOH) or primary amine ($–NH_2$) groups. Magnetic microspheres are supplied at 10% solids (w/w) with added surfactant– sodium dodecyl sulfate (SDS) at 5g/liter– for long-term storage stability.

Proteins can be attached to the surface either by adsorption or by covalent linkage through the COOH, NH_2 or other chemical groups. In all cases, the SDS may be removed by repeated washings. (Ask for TechNotes #13a and #13c for adsorption and covalent coupling protocols.)

Magnetic Properties

All of these microspheres are actually superpara-magnetic, meaning that they have neither magnetic remanence nor hysteresis, as shown in Figure 1. In other words, they respond to a magnetic field, but completely demagnetize when the field is removed. Thus, the microspheres can be easily separated from the liquid phase with a small magnet, but can be redispersed without clumping, immediately after the magnet is removed.

Our ~1µm magnetic microspheres offer the advantage of large specific surface area with a non-porous structure. Without a magnetic field, no significant sedimentation of well-redispersed particles occurs for several hours. With a small magnet the microspheres settle very rapidly, yielding a supernatant that is perfectly transparent. This characteristic is particularly valuable for ELISA tests, where colorimetric determination is performed on the aqueous phase. Figure 2 shows turbidity measurements made on the supernatant of a dilute magnetic microsphere suspension, both with and without a magnet, as a function of settling time and iron oxide content. Due to their small size, the microspheres are kept in motion by thermal agitation and hence no stirring is needed during incubation.

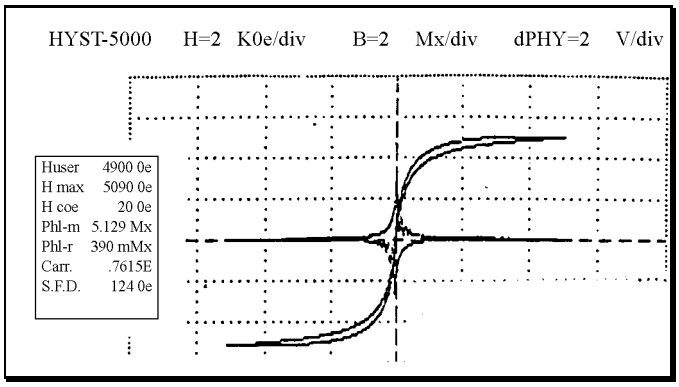


Fig. 1 - Magnetization Curve
22% Magnetite Classical Microspheres
saturation magnetization: 13.2 emu/g
residual magnetization: 1.0 emu/g

Microsphere Handling

Surfactant removal and washing are easily accomplished by magnetic separation. At neutral pH, if the SDS concentration is lowered to 1g/liter, particles may tend to aggregate, but they redisperse easily by adding another surfactant (ANTAROX CO 897, TRITON X 100, TWEEN 20) to the protein solution used for coating.

Note that SDS can be completely removed by washing at pH 9-10 with dilute KOH or NaOH to

open up COO^- surface groups, which will assist in stabilizing the microsphere suspension.

If the microspheres are not stabilized by protein or by a non-ionic emulsifier, then high ionic strength buffers should be avoided, to prevent irreversible agglomeration. Once coated with protein, the microspheres can be used in a variety of buffers, and they may be freeze-dried.

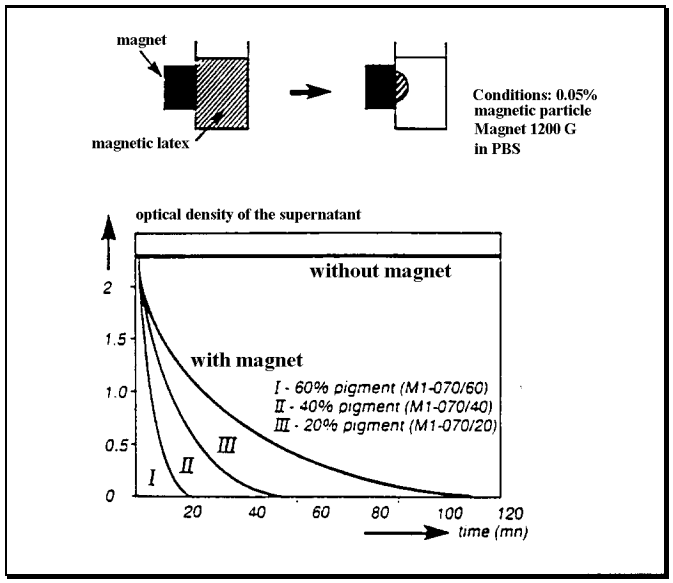


Fig. 2 - Magnetic Clearing - Classical Microsphere Suspensions - 20-60% Fe_3O_4

Storage Conditions

We strongly recommend that our estapor® superparamagnetic microspheres be stored at 4-8°C, while being continuously rolled at a speed of 3-5 revolutions per minute. The storage temperature of 4-8°C reduces microbial growth, while the constant rolling during storage prevents settling. The rolling speed is intended to be fast enough to keep the microspheres suspended, but not so fast that foam is generated in the material.

In the event that constant rolling is not possible, and microspheres become settled in the bottom of the container, we recommend thorough resuspension before use. Our experience indicates that higher speed rolling (30-60 rpm, for at least 4 hours) is effective at resuspending settled material. Again, the rolling speed is intended to be fast enough to resuspend the settled microspheres, but not so fast that foam is generated in the material. Sonication may be used, but is sometimes not effective with large volumes.

Please note that estapor® superparamagnetic microspheres do not contain a preservative, and therefore, should be handled using sterile technique.

Pre-Coated Magnetic Microspheres

In addition to the estapor® range of superparamagnetic microspheres, we supply our *ProActive®* line of protein coated microspheres. Please request Tech. Note #51 for a complete description of our *ProActive®* streptavidin, Protein A and secondary antibody coated microspheres.

Useful References

Magnetic Particle Immunoassay: See V.D.Shah, et al.,"Myocardial Infarction Immunoassay", Eur. Pat Appl. # 91401996.3 (filed 17.07.91). Describes coating (both adsorption and covalent coupling) of Ab onto our magnetic particles and use in immunoassays for CK isoforms. [We are the exclusive US source for these magnetic particles.]

Magnetic Particles Separation Calculations: See K.S. Sachar, B. Goldstein, "Optimization of the Controlled Separation of Biologically Active Diagnostic Magnetic Probes", Computers in Physics, 837-844 (Nov/Dec 1990). Helpful computer model for designing magnetic particle separation systems.

11/13/97

estapor® SUPERPARAMAGNETIC MICROSPHERES

	Stock Number	% Mag.	Polymer Description†		Nominal Mean Diameter (µm)	Polymer Density (g/cm³)	Surface Groups	Surf. Charge (µeq/g)	Parking Area (sq.Å/grp)	Product Class	Price Sch.
	M0008203CN	61%	P(S/6%DVB/V-COOH)Mag		0.82	2.037	COOH/1	163	4	Std	P
			Classical								
	M0011700CN	12%	P(S/V-COOH) Mag.		1.17	1.158	COOH/1			Std	M
	M0011301CN	12%	P(S/V-COOH) Mag.		1.13	1.160	COOH/1			Std	M
	M0011302CN	13%	P(S/V-COOH) Mag.		1.13	1.166	COOH/1			Std	M
	M0010400CN	13%	P(S/V-COOH) Mag.		1.04	1.173	COOH/1			Std	M
•	M0012600CN	13%	P(S/V-COOH)Mag.		1.26	1.173	COOH/1	24	28	Std	M
	M0004600CN	20%	P(S/V-COOH)Mag		0.46	1.248	COOH/1	142	12	Std	M
•	M0015800CN	24%	P(DVB/V-COOH)Mag		1.58	1.292	COOH/1	52	9	Rsch	M
•	M0009601CN	24%	P(DVB/V-COOH)Mag		0.96	1.293	COOH/1	69	12	Std	M
	M0009102CN	24%	P(DVB/V-COOH)Mag		0.91	1.297	COOH/1	64	13	Std	M
•	M0008701CN	25%	P(DVB/V-COOH)Mag		0.87	1.308	COOH/1	53	17	Std	M
•	M0010900CN	25%	P(DVB/V-COOH)Mag		1.09	1.309	COOH/1	58	12	Std	M
	M0009501CN	35%	P(S/60%DVB/V-COOH)Mag		0.95	1.457	COOH/1	83	9	Std	P
	M0006400CN	35%	P(S/60%DVB/V-COOH)Mag		0.64	1.461	COOH/1	86	12	Std	P
	M0007900CN	35%	P(S/60%DVB/V-COOH)Mag.		0.79	1.461	COOH/1	80	11	Std	
	M0010000CN	36%	P(S/60%DVB/V-COOH)Mag		1.00	1.469	COOH/1	81	8	Std	P
	M0013002CN	38%	P(S/60%DVB/V-COOH)Mag		1.30	1.495	COOH/1	92	6	Std	P
•	M0006800CN	38%	P(S/60%DVB/V-COOH)Mag		0.68	1.504	COOH/1	86	11	Std	P
	M0009101CN	38%	P(S/60%DVB/V-COOH)Mag		0.91	1.504	COOH/1	80	9	Std	P
•	M0024600AN	39%	P(S/60%DVB/V-COOH/NH2)Mag		2.46	1.521	COOH&NH2	57	5	Rsch	P
	M0008402CN	40%	P(S/60%DVB/V-COOH)Mag		0.84	1.530	COOH/1	87	9	Std	P
•	M0026000AN	40%	P(S/60%DVB/V-COOH/NH2)Mag		2.60	1.539	COOH&NH2	61	4	Rsch	P
•	M0007000CN	41%	P(S/60%DVB/V-COOH)Mag		0.70	1.560	COOH/1	105	9	Std	P
•	M0008007CN	42%	P(S/60%DVB/V-COOH)Mag		0.80	1.568	COOH/1	97	8	Std	P
	M0009403CN	42%	P(S/60%DVB/V-COOH)Mag		0.94	1.575	COOH/1	80	8	Std	P
	M0007402CN	60%	P(S/6%DVB/V-COOH)Mag	§	0.74	2.006	COOH/1	142	5	Std	P
•	M0007500CN	60%	P(S/6%DVB/V-COOH)Mag	§	0.75	2.006	COOH/1	175	4	Std	P
•	M0007701CN	60%	P(S/6%DVB/V-COOH)Mag		0.77	2.006	COOH/1	150	4	Std	P
•	M0007901CN	60%	P(S/6%DVB/V-COOH)Mag	§	0.79	2.006	COOH/1	145	4	Std	P
	M0009700CN	60%	P(S/6%DVB/V-COOH)Mag		0.97	2.006	COOH/1	100	5	Std	P
	M0009605CN	60%	P(S/6%DVB/V-COOH)Mag		0.96	2.015	COOH/1	152	3	Std	P
	M0007403CN	61%	P(S/6%DVB/V-COOH)Mag		0.74	2.021	COOH/1	98	7	Std	P
	M0008401CN	61%	P(S/6%DVB/V-COOH)Mag	§	0.84	2.037	COOH/1	161	4	Std	P
	M0012400CN	61%	P(S/6%DVB/V-COOH)Mag		1.24	2.037	COOH/1	117	3	Std	P
	M0013001CN	63%	P(S/6%DVB/V-COOH)Mag		1.30	2.085	COOH/1	160	2	Std	P
•	M0009000CN	63%	P(S/6%DVB/V-COOH)Mag	§	0.90	2.115	COOH/1	131	4	Std	P
	M0008201CN	65%	P(S/6%DVB/V-COOH)Mag	§	0.82	2.170	COOH/1	151	4	Std	P
•	M0009400AN	65%	P(S/6%DVB/V-COOH/NH2)Mag		0.94	2.170	COOH&NH2	244	2	Rsch	P
•	M0007500AN	67%	P(S/6%DVB/V-COOH/NH2)Mag		0.75	2.244	COOH&NH2	94	6	Rsch	P
			Encapsulated								
	M0012700CN	13%	P(S/V-COOH)Mag/Encapsulated		1.27	1.170	COOH/1	10	67	Std	N
	M0020300CN	21%	P(S/V-COOH)Mag/Encapsulated		2.03	1.259	COOH/1	40	10	Std	N
•	M0012001CN	21%	P(S/V-COOH)Mag/Encapsulated		1.20	1.260	COOH/1	53	12	Std	N
	M0022900CN	22%	P(S/V-COOH)Mag/Encapsulated		2.29	1.272	COOH/1	21	16	Std	N
	M0015900CN	23%	P(S/V-COOH)Mag/Encapsulated		1.59	1.290	COOH/1	51	10	Std	N
	M0017400CN	24%	P(S/V-COOH)Mag/Encapsulated		1.74	1.292	COOH/1	26	17	Std	N
•	M0009803CN	26%	P(S/V-COOH)Mag/Encapsulated		0.98	1.317	COOH/1	69	11	Std	N

• Immediate Stock Availability

†See Legend and Price Schedule

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	Stock Number	% Mag.	Polymer Description†	Nominal Mean Diameter (µm)	Polymer Density (g/cm^3)	Surface Groups	Surf. Charge (µeq/g)	Parking Area (sq.Å /grp)	Prod-uct Class	Price Sch.
•	M0009804CN	27%	P(S/V-COOH)Mag/Encapsulated	0.98	1.330	COOH/1	72	11	Std	N
	M0015901CN	27%	P(S/V-COOH)Mag/Encapsulated	1.59	1.341	COOH/1	16	29	Std	N
	M0014000CN	38%	P(S/V-COOH)Mag/Encapsulated	1.40	1.509	COOH/1	47	10	Std	N
•	M0008301CN	39%	P(S/V-COOH)Mag/Encapsulated	0.83	1.518	COOH/1	13	61	Std	N
•	M0011300CN	39%	P(S/V-COOH)Mag/Encapsulated	1.13	1.523	COOH/1	53	11	Std	N
•	M0006500CN	40%	P(S/V-COOH)Mag/Encapsulated/Fractionate	0.65	1.541	COOH/1	70	14	Rsch	N
•	M0014300AN	41%	P(S/V-COOH/NH2)Mag/Encapsulated	1.43	1.557	COOH&NH2	177	3	Rsch	N
•	M0010901CN	41%	P(S/V-COOH)Mag/Encapsulated	1.09	1.561	COOH/1	73	8	Std	N
	M0013400CN	41%	P(S/V-COOH)Mag/Encapsulated	1.34	1.561	COOH/1	54	9	Std	N
	M0010500CN	42%	P(S/V-COOH)Mag/Encapsulated	1.05	1.568	COOH/1	49	12	Std	N
	M0011200CN	43%	P(S/V-COOH)Mag/Encapsulated	1.12	1.589	COOH/1	89	6	Std	N
			Narrow Distribution							
•	M0006000CN	20%	P(S/V-COOH)Mag/Narrow	0.60	1.248	COOH/1	55	24	Rsch	N
•	M0006700CN	20%	P(S/V-COOH)Mag/Narrow	0.67	1.248	COOH/1	53	22	Rsch	N
•	M0005900CN	40%	P(S/V-COOH)Mag/Narrow	0.59	1.539	COOH/1	95	12	Rsch	N

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Abbreviation Legend

11/13/97

Polymer or Abbreviation	Meaning
A	Acrylic polymer, unspecified
AA	Acrylic Acid
Ar	Aromatic (benzene-ring linked)
B	Butadiene
BMA	ButylMethAcrylate
BrS	BromoStyrene
CH2-Cl	Chloromethyl group
CH2-OH	Alcohol (hydroxyl group)
CHO	Aldehyde group
CONH2	Amide group or monomer
COOH	Carboxylic Acid Group
COOH/1	COOH/Type 1: S/AA copolymer
COOH/2	COOH/Type 2: S/MA copolymer
COOH/4	COOH/Type 4: Type 1 + spacer arm
COOH/5	COOH/Type 5: copolymer with spacer arm ~10Å
DVB	DiVinylBenzene
EDA	EthyleneDiAmine
EDMA	EthyleneDiMethAcrylate
EMA	EthylMethAcrylate
Fl.	Fluorescent
GMA	GlycidylMethAcrylate
MA	Maleic Anhydride
MAA	MethAcrylic Acid
Mag.	Magnetite - Fe_3O_4
MMA	MethylMethAcrylate
MS	MethylStyrene
NHNH2	Hydrazide group
P( )	Poly(copolymer)
PMMA	PolyMethylMethAcrylate
pMS	paraMethylStyrene
PS	PolyStyrene
PVAc	PolyVinylAcetate
PVT	PolyVinylToluene
R	Aliphatic
Rsch	Research material
S	Styrene
SO3	Sulfonic acid groups
Std	Standard Material - replicate lots available.
V	Vinyl, unspecified polymerizable group
VBC	VinylBenzylChloride
VdCl	Vinylidene Chloride
VT	VinylToluene
XL	Cross-linked
__%	percentage of magnetite or monomer used
§	Anti microbial present (Sodium Azide or Merthiolate)
•	Before Stock no.= Immediate availability