

Sphero TECHNICAL NOTES

STN-10 REV A 1/98

**Magnetic Particle Enzyme Immunoassay (MPEIA) Test Procedure using
UltraMag Separator System (UMS-4000)**

NOTE: Specific procedure for MPEIA will vary depending on the purpose and nature of the assay. Following is a general description of a typical test, however, suitable modification should be made to suit specific assay requirements.

1. Make serial 2 fold dilutions of rabbit, human or mouse IgG in 1% bovine serum albumin (BSA) in wells of microEIA plate; 100 μ l /well.

NOTE: Control wells in column 11 and 12 receive 100 μ l of 1% BSA without IgG.

2. Add 50 μ l of 0.25% (w/v) suspension of Spherotech CM 40 magnetic particles coated with antibodies to rabbit, human or mouse IgG. Particles are diluted in buffer consisting of 1% casein hydrolysate and 0.05% Tween 20 in phosphate buffer saline, pH 7.2.
3. Cover microEIA plate and incubate it for 15-30 minutes at room temperature.
4. Place microEIA plate on *UltraMag Separator* (P/N 539-1401A) in a way that magnetic pegs fit in the spaces between wells of microEIA plate.
5. Wash magnetic particles 3 to 5 times by repeating cycles of magnetic particle separation (1 to 3 minutes), aspiration and adding wash solution (200 μ l/well).

Note: Particles may be resuspended between cycles by tapping after adding wash solution.

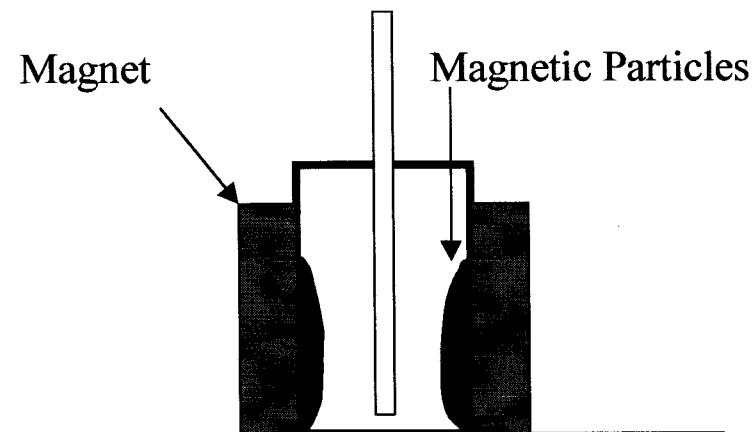
6. Add 100 μ l of conjugate (antibodies to rabbit, human or mouse IgG conjugated to alkaline phosphate) to each well.
7. Cover microEIA plate and incubate it for 15-30 minutes at room temperature.
8. Separate and wash particles as in steps 4 and 5.
9. Add substrate (Sigma N-1891), 100 μ l per well.
10. Mix and incubate for 15-30 minutes at room temperature.
11. Place the microEIA plate on *UltraMag Separator* (P/N 539-1401A) as described in step 4 above.
12. After a 2 minute separation, insert microEIA plate set atop the *UltraMag Separator* into a microEIA plate reader (e.g. MRX by Dynex Laboratories).

Spherotech, Inc.

Why MPEIA?

There are several advantages of MPEIA over coated well EIA; some of them are listed below:

1. Reaction kinetics of small uniform magnetic particles added to a sample solution is fast and efficient. Kinetics for coated well plate EIA are significantly slower because only the layer of solution directly in contact with the coated surface has any chance of interaction with the analytes in solution.
2. Removal of unbound reactants is more thorough with uniform magnetic particles because all sides of the particles are washed when in suspension. Ability to remove most of the unbound reactants helps to achieve lower non specific background which in turn improves assay sensitivity.
3. Magnetic particles can be coated in very small to very large batches ensuring uniformity of coating for a large number of tests. Uniform coating allows delivery of particles with similar properties to all wells. In contrast, coated well plate EIA often shows significant well to well variation as well as margination effect since each well is coated individually.
4. Surface properties of magnetic particles can be modified to maximize and / or orient molecules attached to its surface. Various molecules can be attached to the particle surface by passive adsorption or by covalent linkage as necessary.
5. Only magnetic particles can capture specific analytes in a suspension ignoring other components in solution; thereby concentrating it in the pellet within a few minutes without centrifugation. The magnetic particle pellet with the captured analytes can be transferred to the well of the microEIA plate for analysis.
6. With the development of *UltraMag Separator System* (UMS-4000) magnetic particle loss during washing has been eliminated as illustrated below.



Magnetic Particles Wash using UltraMag Separator

BIBLIOGRAPHY

Selected references showing use of magnetic particles in immuno assays.

1	Bennick A, Brosstad F Immunomagnetic separation and solid-phase detection of <i>Bordetella pertussis</i> . J Clin Microbiol 1996; 34: 778-784
2	Hayes MC, Jourdan SW, Herzog DP Determination of atrazine in water by magnetic particle immunoassay: collaborative study. J AOAC Int 1996; 79: 529-537
3	Horton J K et al. A new and rapid method for the selection and cloning of antigen specific hybridomas with magnetic microspheres. J Immunol Methods 1989; 124: 225-230
4	Kala M, Bajaj K, Sinha S Magnetic bead enzyme-linked immunosorbent assay (ELISA) detects antigen- specific binding by phage-displayed scFv antibodies that are not detected with conventional ELISA. Anal Biochem 1997; 254: 263-266
5	Lawruk TS, Hottenstein CS, Herzog DP, Rubio FM Quantification of alachlor in water by a novel magnetic particle-based ELISA. Bull Environ Contam Toxicol 1992; 48: 643-650
6	Leahy, D., Shah, D., Arima, T., et al. Improved serological detection of Hepatitis C Virus with a Paramagnetic Microparticle Assay using multiple antigen sequences. Transfusion 1992; 32: 548
7	Lim PL A one-step two-particle latex immunoassay for the detection of <i>Salmonella typhi</i> endotoxin. J Immunol Methods 1990; 135: 257-261.
8	Lim PL, Ko KH A tube latex test based on colour separation for the detection of IgM antibodies to either one of two different microorganisms. J Immunol Methods 1990; 135: 9-14
9	Lim PL, Ko KH A tube latex test based on colour separation for the detection of IgM antibodies to either one of two different microorganisms. J Immunol Methods 1990;135: 9-14.
10	Lim PL, Ko KH, Choy WF A two-particle turbidometric latex immunoassay for the detection of specific IgM antibodies.

	J Immunol Methods 1989; 117: 267-273
11	Nakamura N, Hashimoto K, Matsunaga T Immunoassay method for the determination of immunoglobulin G using bacterial magnetic particles. Anal Chem 1991; 63:268-272
12	Nakamura N, Hashimoto K, Matsunaga T Immunoassay method for the determination of immunoglobulin G using bacterial magnetic particles. Anal Chem 1991; 63: 268-272
13	Nath, N. et al. Stability of the recombinant hepatitis B core antigen. J Clin Microbiol. 1992; 30: 1617-1619.
14	Obenauer-Kutner LJ, Jacobs SJ, Kolz K, Tobias LM, Bordens RW A highly sensitive electrochemiluminescence immunoassay for interferon alfa-2b in human serum. J Immunol Methods 1997; 206: 25-33
15	Ossendorp F A et al. Efficient selection of high affinity B cell hybridomas using antigen- coated magnetic beads. J Immunol Methods. 1989; 120: 191-200
16	Rossomomando, E.F. et al. Immunomagnetic separation of tumor necrosis factor alpha . I. Batch procedure for the human tempromandibular fluid. J Chromatogr. 1992; 583: 11-18.
17	Rossomomando, E.F. et al. Immunomagnetic separation of tumor necrosis factor alpha. II. In situ procedure for the human gingival space. J Chromatogr. 1992; 583:19-26.
18	Stark M, Reizenstein E, Uhlen M, Lundeberg J A rapid method for selecting specific hybridoma clones using paramagnetic Dynabeads. Scand J Immunol 1993; 38: 212-214
19	Stark M, Reizenstein E, Uhlen M, Lundeberg J Immunomagnetic separation and solid-phase detection of Bordetella pertussis. J Clin Microbiol 1996; 34: 778-784
20	Todd J., Kink, J, Shah, D. et al. A novel semi automated paramagnetic microparticle based enzyme immunoassay for hepatitis C virus: its application to serological testing. J Immunoassay. 1992; 13: 393-410.
21	Ugelstad J, Stenstad P, Kilaas L, Prestvik WS, Herje R, Berge A, Hornes E Monodisperse magnetic polymer particles. New biochemical and biomedical applications. Blood Purif 1993; 11: 349-369
22	Ushijima H, Honma H, Tsuchie H, Kitamura T, Takahashi I Removal of HIV antigens and HIV-infected cells in vitro using immunomagnetic beads. J Virol Methods 1990; 29:23-31

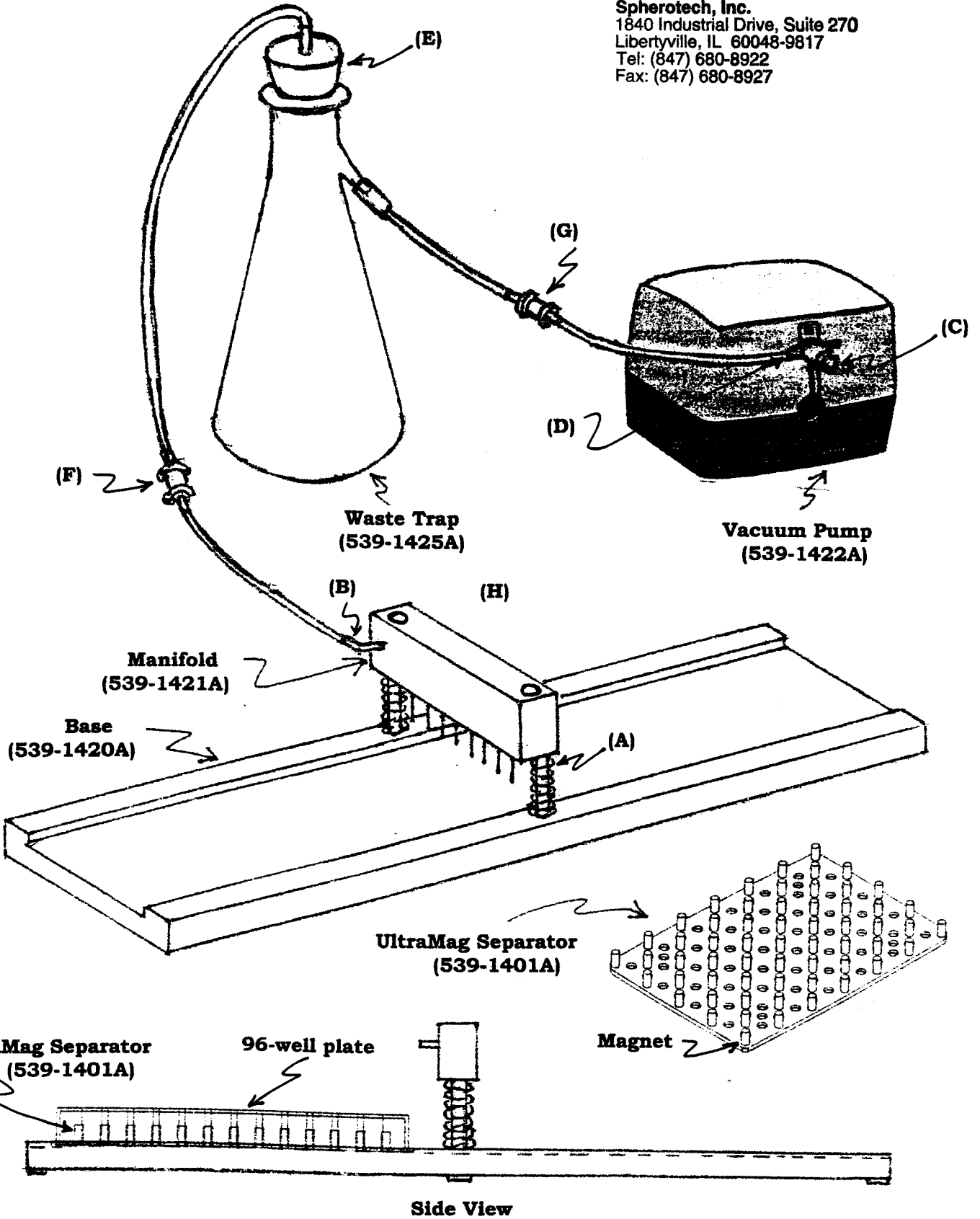
Spherotech, Inc.

1840 Industrial Dr., Suite 270, Libertyville, IL 60048

Phone: 800-368-0822 or 847-680-8922; Fax: 847-680-8927; E-mail: jwsphero@aol.com

Visit us on the web at <http://www.spherotech.com>

Spherotech, Inc.
1840 Industrial Drive, Suite 270
Libertyville, IL 60048-9817
Tel: (847) 680-8922
Fax: (847) 680-8927



UltraMag Separator System
(Cat# UMS-4000)