SPHERO™ Technical Note **STN-10 Rev B 081307**

Magnetic Particle Enzyme Immunoassay (MPEIA) Test Procedure using the Spherotech UltraMag Separator (Cat. No. UMS-3000)

INTRODUCTION

Magnetic Particle Enzyme Immunoassay (MPEIA) is an immunoassay method for the isolation of antibody/antigen complexes on a solid phase surface of magnetic microparticles. MPEIA has been used to automate the measurement of large molecules such as markers associated with cardiac, fertility, cancer, metabolic, hepatitis, and thyroid testing.

PROCEDURE

1. In the wells of a microEIA plate make serial 2 fold dilutions of rabbit, human or mouse IgG in 1% bovine serum albumin (BSA) adding 100 µL /well. The control wells should receive 100 µL of 1% BSA without IgG.

2. Add 50 µL of a 0.25% (w/v) suspension of Spherotech Cat. No. CM-40-10 magnetic particles coated with antibodies to rabbit, human or mouse IgG. Dilute the particles in buffer consisting of 1% casein hydrolysate and 0.05% Tween 20 in phosphate buffer saline, pH 7.2.

3. Cover the microEIA plate and incubate for 15-30 minutes at room temperature.

4. Place the microEIA plate on the UltraMag Separator (Cat. No. UMS-3000) so that the magnetic pegs fit in the spaces between wells of the plate.

5. Wash the magnetic particles 3 to 5 times by repeating cycles of magnetic particle separation (1 to 3 minutes), aspiration and adding wash solution (200uL/well). The particles may be resuspended between cycles by tapping after adding wash solution.

6. Add 100 µL of conjugate (antibodies to rabbit, human or mouse IgG conjugated to alkaline phosphate) to each well.

7. Cover the microEIA plate and incubate it for 15-30 minutes at room temperature.

8. Separate and wash the particles as in steps 4 and 5.

9. Add the substrate (Sigma Cat. No. N1891, SIGMAFAST™ p-Nitrophenyl phosphate), 100 µL per well.

10. Mix and incubate for 15-30 minutes at room temperature.

11. Place the microEIA plate on the UltraMag Separator as described in step 4 above.

12. After 2 minutes of separation, insert the microEIA plate atop the UltraMag Separator into a microEIA plate reader (e.g. MRX by Dynex Laboratories).

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

Why MPEIA?
Several advantages of MPEIA over coated well EIA are listed below:

1. The reaction kinetics of small uniform magnetic particles added to a sample solution is fast and efficient. The 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. The removal of unbound reactants is more thorough with uniform magnetic particles because all sides of the particles are washed when in suspension. The ability to remove most of the unbound reactants helps to achieve lower non specific background which in turn improves assay sensitivity.

3. The 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 the 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. The 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. As a result, the analytes can be concentrated 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.

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