

PolyPlex SP Microspheres: Goat Anti-Mouse, Streptavidin, and Carboxyl

PolyPlex SP is a single population of microspheres sized 4.4 micron or 5.5 micron. The beads are internally dyed with Starfire Red™ fluorescent dye that is fluorescent in FL3 and are excitable at 488 nm. The beads have a uniform Goat anti-Mouse IgG (GAM), Streptavidin or carboxyl (COOH) surface. The GAM surface allows easy attachment of mouse-derived antibodies; the COOH surface allows for the easy covalent attachment of analytes or analyte-specific antibodies and the streptavidin surface is used for biotinylated antibodies or other biotinylated molecules. In each of these cases, the beads may then be incubated with a sample and washed before a fluorescently tagged reporter antibody is added. After a second wash and resuspension, the beads may be analyzed with a flow cytometer to determine the presence or absence of the assayed analyte.

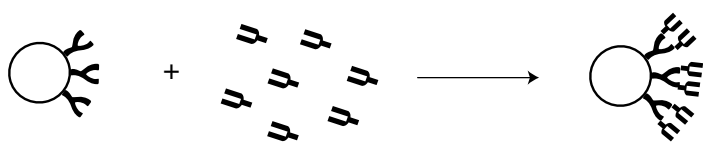
PolyPlex SP microspheres are bottled individually in 1mL or 3mL aliquots at a concentration of approximately 1.0×10^6 microspheres/mL. They are provided in a buffered solution containing 0.1% BSA, 0.05% Tween 20, and 10mM EDTA.

Procedure

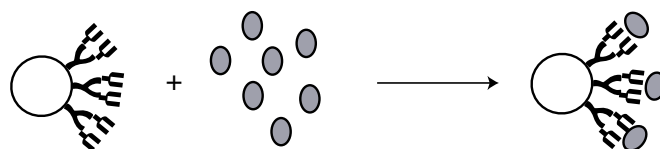
PolyPlex SP allows for flexibility in designing individual experiments. The preparation procedures outline the conjugation of a single antibody to a PolyPlex SP bead. In doing so, an assay may be produced which is capable of testing a single sample for a single analyte. The user may then choose instead to conjugate multiple antibodies of different specificities to the beads, producing an assay ideal for screening a sample for multiple analytes in a single test. The user may further choose to conjugate antigen to the beads, yielding an assay capable of testing for the presence of specific antibody. The specific application is to be determined by the user.

NOTE: The following outline serves as a guide, and may be modified to reflect the user's specific application.

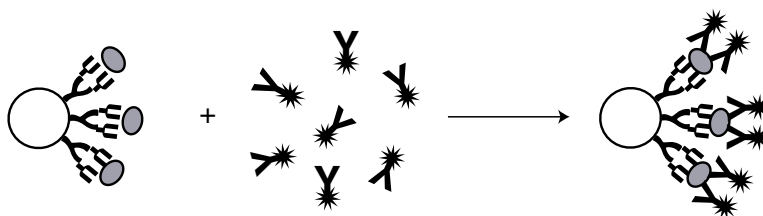
Schematic of Procedure using PolyPlex SP Goat Anti-Mouse Beads:



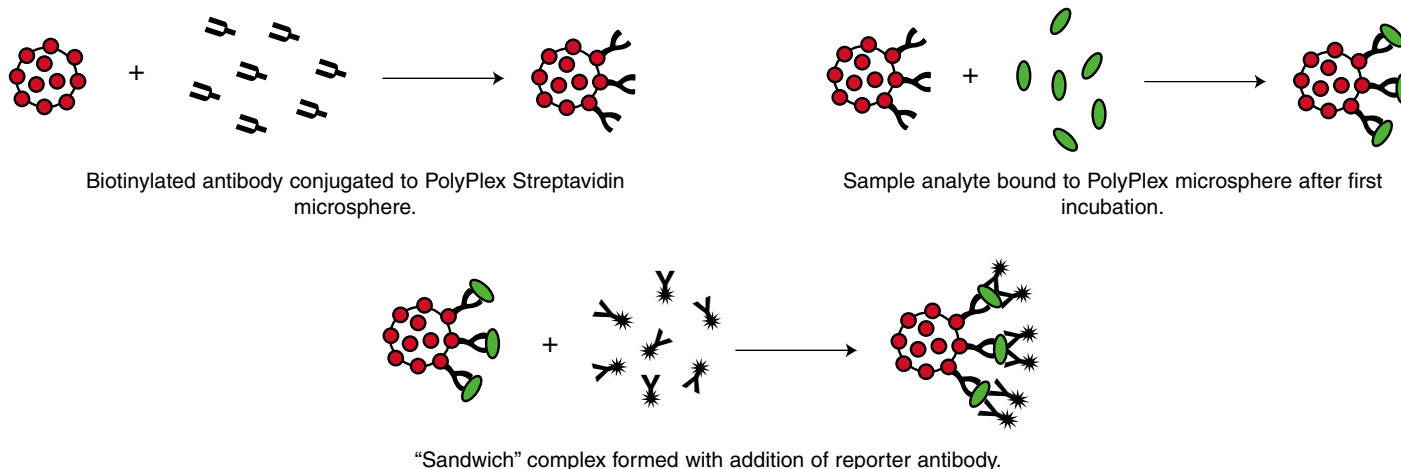
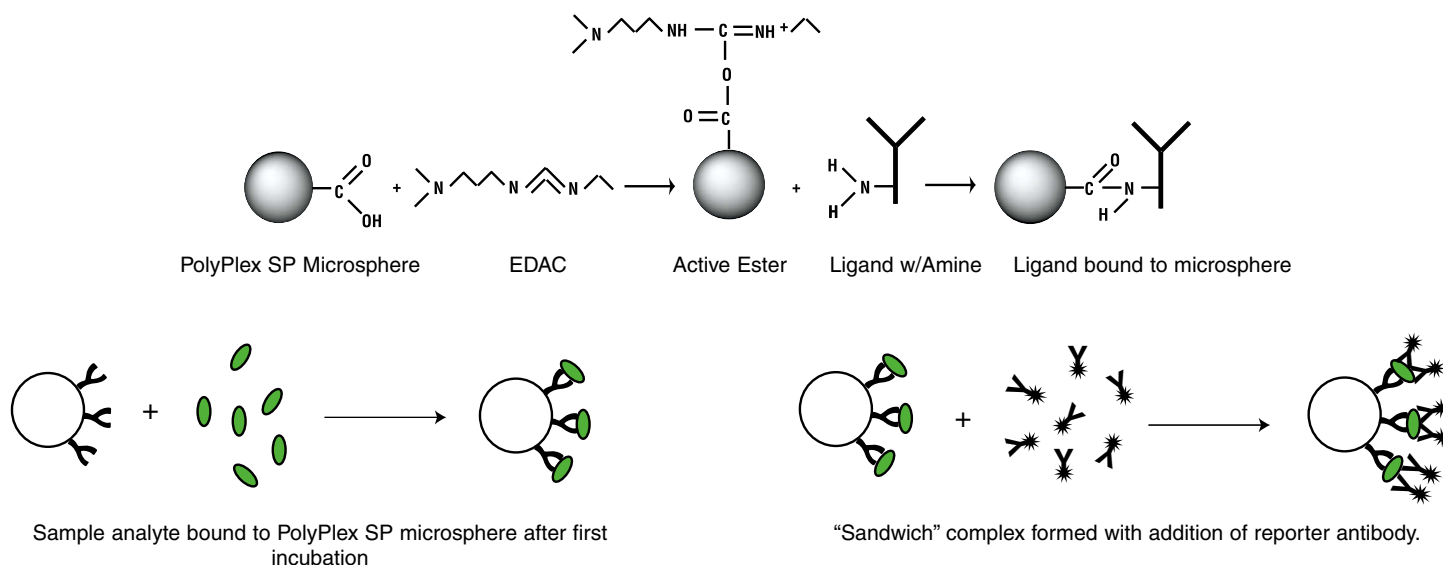
Mouse antibody conjugated to PolyPlex GAM microsphere



Sample analyte bound to PolyPlex microsphere after first incubation.



"Sandwich" complex formed with addition of reporter antibody.

Schematic of Procedure using PolyPlex SP Streptavidin Beads:**Schematic of Procedure using PolyPlex SP Carboxyl Beads:****Preparation of Microspheres**

1. Vortex the bottle prior to use to ensure uniform suspension of beads.
2. Immediately remove 10 μ L of solution to be labeled with ligand.

NOTE: The ten microliter volume reflects the amount needed to conduct one test using the given bead population. For ease of use, the entire 1 mL or 3 mL may be labeled all at once, and then stored for use with each test.

3. Using an excess of biotinylated antigen or antibody, conjugate the antigen or antibody to the bead using established protocols. (One approach is to simply incubate the beads and the mouse antibody together for 30 minutes.)
4. Wash the beads to remove any unbound antibody.
5. Resuspend beads in ~100 μ L buffer.

Testing Samples

1. Incubate prepared beads with 100 μ L sample(s) for 30 minutes. (The volume of sample used may be adapted to the specific application.)
2. Wash beads to remove non-specifically bound analyte. Repeat the wash step.
3. Incubate the beads with 20 μ L of the appropriate fluorescently labeled antibody for 30 minutes.
4. Wash beads to remove non-specifically bound antibody. Repeat the wash step.
5. Acquire data events using a flow cytometer.

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Data Analysis

1. Gate on the singlet population(s) on a Forward Scatter vs. Side Scatter plot.
2. Using the FL1 and/or FL2 channels (depending on the reporter antibodies used) determine whether or not any bead populations tested "positive" for the analyte.

NOTE: A positive bead will produce a fluorescence peak in the FL1 or FL2 channel. The minimum fluorescence intensity needed to be considered "positive" is based on the Relative Channel Value of the peak. It is up to the investigator to determine what threshold RCV value will constitute a "positive" result.

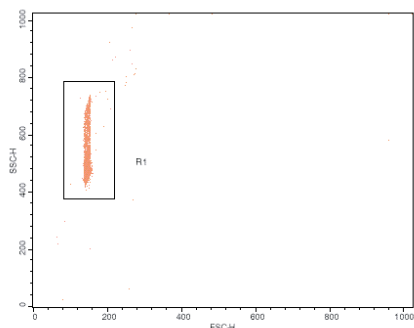


Figure 1.

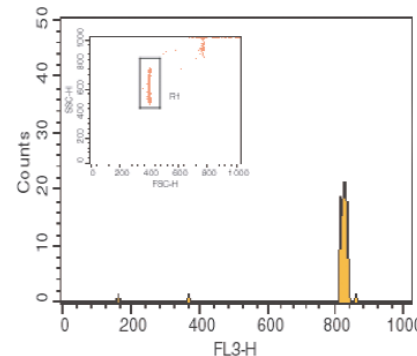
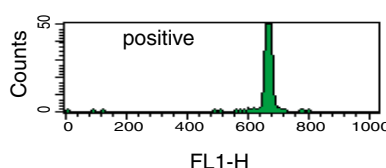
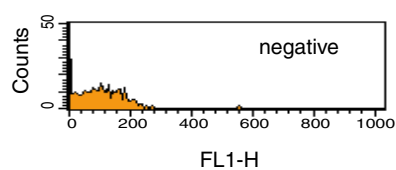


Figure 3.

Storage and Handling:

PolyPlexSP beads are stable for twelve (12) months from the date of opening when handled in accordance with the manufacturer's recommendations. The beads should be kept in the bottle in which they are shipped, and stored at 2-8°C. Do not expose the beads to intense light sources for extended periods of time, and DO NOT FREEZE the beads.

PolyPlexSP beads are suspended in a buffered solution containing the preservative sodium azide (0.1%). Under acidic conditions, sodium azide forms hydrazoic acid, an extremely toxic compound. To avoid deposits in plumbing where explosive conditions may develop, dilute with running water when discarding.

Ordering Information:

Catalog #	Description	Size
24340-1	PolyPlex SP Anti-Mouse IgG, 4.4µm	1ml
24340-3	PolyPlex SP Anti-Mouse IgG, 4.4µm	3ml
24341-1	PolyPlex SP Anti-Mouse IgG, 5.5µm	1ml
24341-3	PolyPlex SP Anti-Mouse IgG, 5.5µm	3ml
24342-1	PolyPlex SP Carboxyl, 4.4µm	1ml
24342-3	PolyPlex SP Carboxyl, 4.4µm	3ml
24343-1	PolyPlex SP Carboxyl, 5.5µm	1ml
24343-3	PolyPlex SP Carboxyl, 5.5µm	3ml
24344-1	PolyPlex SP Streptavidin, 4.4µm	1ml
24344-3	PolyPlex SP Streptavidin, 4.4µm	3ml
24345-1	PolyPlex SP Streptavidin, 5.5µm	1ml
24345-3	PolyPlex SP Streptavidin, 5.5µm	3ml

To Order:

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In The U.S. FAX: 1-800-343-3291 • 215-343-0214

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