

9025 Technology Dr. • Fishers, IN 46038-2886
 800.387.0672 • 317.570.7020 • Fax 317.570.7034
 info@bangslabs.com • www.bangslabs.com

Bangs Laboratories, Inc.

BEADS ABOVE THE REST™

DESCRIPTION

The QuantumPlex™ M kit is designed to be used as a multiplexing platform, allowing for the efficient, qualitative analysis of a sample for multiple analytes, or the high throughput screening of multiple samples. QuantumPlex™ M are suitable for use with any conventional cytometer, and magnetic separations may be performed using a standard laboratory (rare earth) magnetic separation device.

QuantumPlex™ M SP is a single population of ~6µm highly uniform superparamagnetic microspheres internally dyed with Starfire Red™ fluorescent dye (fluorescent in FL3). The beads have a streptavidin (SA) surface, permitting the easy conjugation of biotinylated analytes or analyte-specific antibodies. The beads are then incubated with a sample and washed before a fluorescently-tagged reported antibody is added. After a second wash and resuspension, the beads may be analyzed with a flow cytometer to determine which samples contained the analyte.

CHARACTERISTICS

Mean Diameter: ~6µm
 Particle Concentration: 1 x 10⁶ microspheres/mL

MATERIAL

Material Supplied

- Superparamagnetic QuantumPlex™ M microspheres: bottled individually in 1 mL or 3 mL aliquots

Material Required

- Biotinylated analyte or antibody specific to the analyte(s) of interest
- Buffered wash solution
- Fluorescently-labeled reporter antibody (fluorescent in FL1 or FL2)

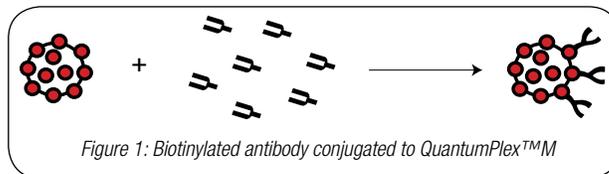
PROCEDURE

Researchers are advised to optimize the use of particles in any application.

The QuantumPlex™ M SP Streptavidin allows for flexibility in designing individual experiments. The preparation procedure outlines the conjugation of a single biotinylated antibody to the QuantumPlex™ M bead population. In doing so, an assay may be produced which is capable of testing a single sample for a single analyte. The user may choose instead to conjugate multiple antibodies of different specificities to the bead, producing an assay ideal for screening a sample for multiple analytes at once. The user may further choose to conjugate antigen to the beads, yielding an assay capable of testing for the presence of a specific antibody. The specific application is to be determined by the user. The following outline serves as a guide, and may be modified to reflect the user's specific application.

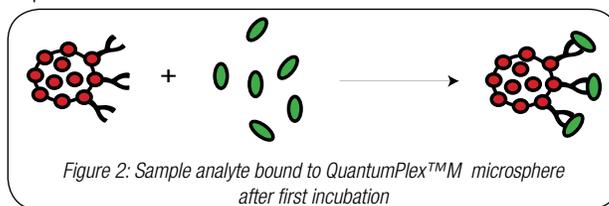
Preparation of Microspheres

1. Vortex each bottle prior to use to ensure uniform suspension of the beads.
2. Immediately remove 10µL of solution to be labeled with ligand. *Note:* The 10µL volume reflects the amount needed to conduct one test using the given bead population. For ease of use, the entire 1 mL may be labeled all at once, and then stored for use with each test.
3. Wash microspheres to remove surfactant and additives from the storage buffer (e.g. wash 3 times in PBS, pH 7.4).
4. Using an excess of biotinylated antigen or antibody, conjugate the antibody or antigen to the bead using established protocols. *Note:* One approach is to simply incubate the beads and the antibody or antigen together, in suitable buffer, for 30-60 minutes. See also TechNote 101, *ProActive® Microspheres*, for sample conjugation protocols.

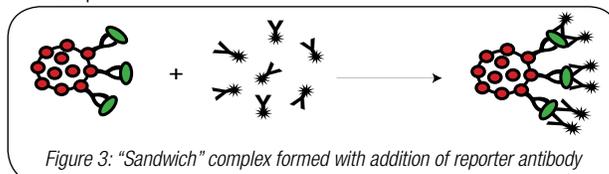


Testing Samples

1. Incubate prepared beads with 100µL sample(s) for 30 minutes. (The volume of sample used may be adapted to the specific application.)
2. Wash beads to remove nonspecifically bound analyte. Repeat the wash step.



3. Incubate the beads with 20µL of the appropriate fluorescently-labeled antibody for 30 minutes.
4. Wash beads to remove nonspecifically bound antibody. Repeat the wash step.



5. Combine all beads in one tube and acquire data events using a flow cytometer.

Data Analysis

1. Gate on the single population(s) on a Forward Scatter vs. Side Scatter plot. (Figure 4)

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. (Figure 5) *Note:* A positive bead will produce a fluorescent peak in the FL1 or FL2 channel. The minimum fluorescence intensity needed to be considered “positive” is based on the Relative Channel Value (RCV) of the peak. It is up to the investigator to determine what threshold RCV value will constitute a “positive” result.
3. Using your flow cytometry analysis software, determine which beads produced positive results. The use of “back-gating” may simplify this task. (Figure 6)
4. Based upon the beads that produced positive results, determine which samples contained the analyte, or which analytes the samples contained.

Figure 4

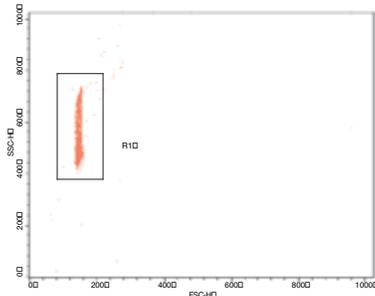


Figure 5

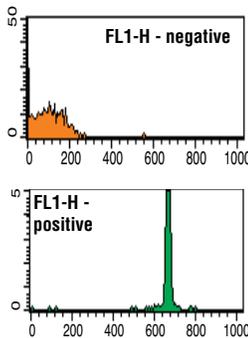
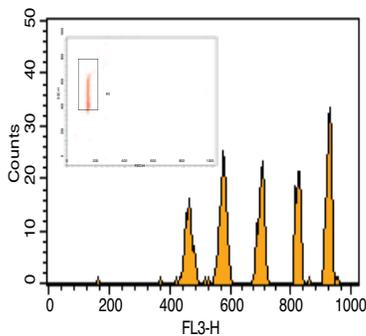


Figure 6



STORAGE AND STABILITY

Store at 2-8°C. Freezing may result in irreversible aggregation and loss of binding activity. QuantumPlex™M beads are stable for 12 months from date of purchase, provided the product is handled in accordance with the manufacturer’s recommendations. The beads should be kept in the bottles in which they are shipped. Do not expose beads to intense light sources for extended periods of time.

SAFETY

This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Sizes
253	QuantumPlex™M SP Streptavidin ~6µm Magnetic	1mL or 3mL

Order online anytime at www.bangslabs.com.