Product Data Sheet 235

QuantumPlex™ Carboxyl

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.

B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

The QuantumPlex™ 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™ kits are available in five-bead sets of microspheres sized 4.4µm (Catalog Code 235), 5.5µm (Catalog Code 238), and in ten-bead sets comprised of the two (Catalog Code 239). Each five-bead set consists of five (5) bead populations internally dyed with varying intensities of Starfire Red™ fluorescent dye (fluorescent in FL3). The beads have a uniform carboxyl (COOH) surface. The COOH surface allows for the easy conjugation of analytes or analyte-specific antibodies to the surface of each bead. The beads may then be 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 the presence or absence of the assayed analyte.

CHARACTERISTICS

Mean Diameter:	4.4µm (Catalog Code 235) or 5.5µm (Catalog Code 238)
Particle Concentration:	1 x 10^8 microspheres/mL

MATERIAL

Material Supplied

- QuantumPlex™ microspheres: bottled individually in 1mL, 5mL, or 10mL aliquots

Material Required

- Analyte or antibody specific to the analyte(s) of interest
- Coupling Buffer: pH 7.2-8.5
- Activation Buffer: pH 4.5-7.5
- Water Soluble Carbodiimide, WSC (EDAC, EDC, CMC, etc.)
- Storage Buffer: 0.01-0.1% (w/v) blocking 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™ kit allows for flexibility in designing individual experiments. The preparation procedure outlines the conjugation of a single antibody to the QuantumPlex™ bead. Conjugating each of the beads to an single antibody specific to a different analyte yields a kit capable of testing a single sample for multiple analytes. The user may choose instead to conjugate multiple antibodies of different specificities to each bead, producing a kit ideal for screening multiple samples at once. The user may further choose to conjugate antigen to the beads, yielding a kit capable of testing for the presence of a specific antibody. The specific application is to be determined by the user. It may be helpful to make a table to keep track of which antibodies have been conjugated to each bead population. The following outline serves as a guide, and may be modified to reflect the user's specific application. For a more detailed coupling procedure, see TechNote 205, *Covalent Coupling*.

Preparation of Microspheres

1. Vigorously shake or vortex the 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 1mL or 3mL may be labeled all at once, and then stored for use with each test.
3. Wash microspheres 2 times with activation buffer, resuspending in same.
4. While mixing, add WSC.
5. Allow to react at room temperature for 15 minutes with continuous mixing.
6. Wash 2 times in coupling buffer, resuspending in same.
7. Dissolve ligand to be coupled (1-10X excess of calculated monolayer. See TechNote 205, *Covalent Coupling*.) in coupling buffer.
8. Combine microsphere solution and ligand solution, and allow to react at room temperature for 2-4 hours with constant mixing.
9. Wash and resuspend in quenching solution, and mix gently for 30 minutes.
10. Wash and resuspend in storage buffer at original concentration.
11. Store at 4˚C until used.

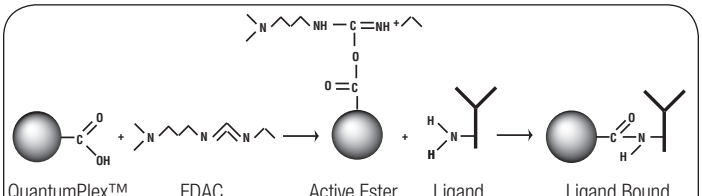


Figure 1: Coupling Procedure

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.

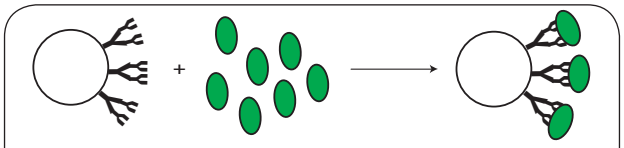


Figure 2: Sample analyte bound to QuantumPlex™ microsphere after first incubation

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.

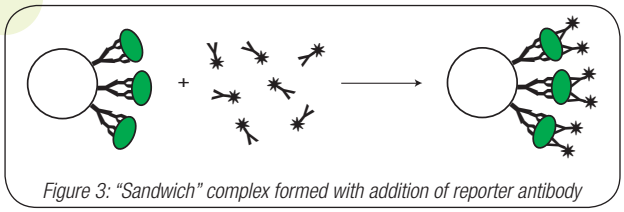


Figure 3: "Sandwich" complex formed with addition of reporter antibody

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

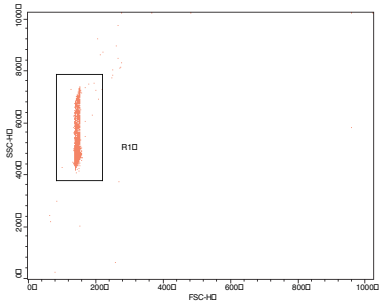


Figure 5

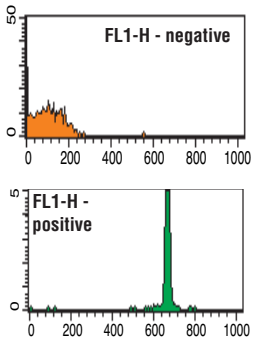
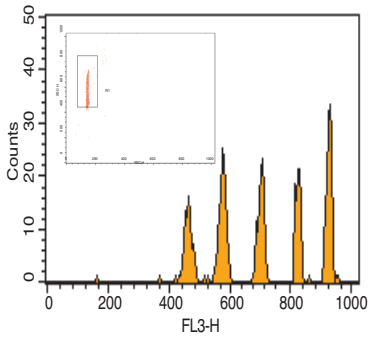


Figure 6



STORAGE AND STABILITY

Store at 2-8˚C. Freezing may result in irreversible aggregation and loss of binding activity. QuantumPlex™ kits 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
235	QuantumPlex™ Carboxyl 4.4µm (5 dye intensities)	1mL, 5mL, or 10mL
238	QuantumPlex™ Carboxyl 5.5µm (5 dye intensities)	1mL, 5mL, or 10mL
239	QuantumPlex™ Carboxyl 4.4µm and 5.5µm (2 x 5 dye intensities)	1mL, 5mL, or 10mL

Order online anytime at **www.bangslabs.com.**