

SPHERO™ Flow Cytometry Multiplex Bead Assay Particles

SPHERO™ Flow Cytometry Multiplex Bead Assay Particles are designed for the development of flow cytometer multiplex assays. These kits are used by companies or laboratories which develop assays for allergy testing, autoimmune diseases, cardiac markers, cytokine detection, endocrine markers, infectious disease markers, isotyping, genotyping, kinase and phosphorylated protein activity, metabolic markers, and tissue typing. For example, S. Chew, et al. "Stability Screening of Arrays of Major Histocompatibility Complexes on Combinatorially Encoded Flow Cytometry Beads." *J. Biol. Chem.* 2011 286: 28466-28475. These kits are available with a variety of functionality, fluorophores and coatings.

SPHERO™ Blue Fluorescent Particle Array Kits (PAK)

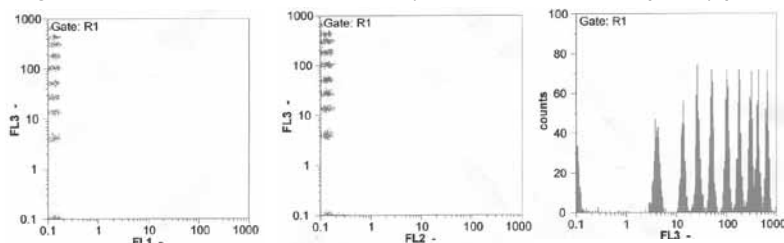
- Designed to simplify flow cytometer multiplex assay development
- Consists of fluorescent particles of different intensities in PE-Cy5 channel and minimal fluorescence in FITC and PE channels with 488 nm excitation
- Used with FITC and/or PE for detection
- Several sizes can be used independently or mixed together (3.5, 4.0 and 5.1 & 7.7 micron sizes available).
- Available as Functionalized, Streptavidin, Biotin and antibody coated.

Blue PAK (**P**article **A**rray **K**its) for multiplex flow assay are now available from Spherotech. The Blue PAK particles are fluorescent in PE-Cy5, APC, and APC-Cy7 channel with minimal fluorescent in FITC and PE channels with 488 or 635 nm excitation. This allows that either FITC and/or PE tracers can be used for detection. These kits are available with different functionalities and coatings. Carboxyl, amino and plain polystyrene functionalized particles are available for covalent attachment or passive adsorption of ligands. The Blue PAK and Carboxyl Blue PAK are available in 7.66 μm (9 peaks), 5.1 μm (10 peaks), 4.0 μm (8 peaks) and 3.6 μm (7 peaks) sizes. Blue PAK particles are also available coated with Streptavidin and Goat anti-Mouse IgG (H&L).

Particle Type and Surface	Size, μm	Catalog No.	Unit
Blue - 7 peaks, 10 ⁸ /mL	3.5-3.9	PAK-3567-7K	7x1 mL
Blue - 8 peaks, 10 ⁸ /mL	4.0-4.9	PAK-4067-8K	8x1 mL
Blue - 10 peaks, 10 ⁸ /mL	5.0-5.9	PAK-5067-10K	10x1 mL
Blue - Odd # peaks, 10 ⁸ /mL	5.0-5.9	PAK-5067-5A	5x1 mL
Blue - Even # peaks, 10 ⁸ /mL	5.0-5.9	PAK-5067-5B	5x1 mL
Blue - 9 peaks, 10 ⁷ /mL	7.0-7.9	PAK-7067-9K	9x1 mL
Carboxyl Blue - 7 peaks, 10 ⁸ /mL	3.5-3.9	CPAK-3567-7K	7x1 mL
Carboxyl Blue - 8 peaks, 10 ⁸ /mL	4.0-4.9	CPAK-4067-8K	8x1 mL
Carboxyl Blue - 10 peaks, 10 ⁸ /mL	5.0-5.9	CPAK-5067-10K	10x1 mL
Carboxyl Blue - Odd # peaks, 10 ⁸ /mL	5.0-5.9	CPAK-5067-5A	5x1 mL
Carboxyl Blue - Even # peaks, 10 ⁸ /mL	5.0-5.9	CPAK-5067-5B	5x1 mL
Carboxyl Blue - 9 peaks, 10 ⁷ /mL	7.0-7.9	CPAK-7067-9K	9x1 mL
Carboxyl Blue Chemistry Development Particles, 10 ⁸ /mL	5.0-5.9	CFP-5067-2	2 mL
Amino Blue - 7 peaks, 10 ⁸ /mL	3.5-3.9	APAK-3567-7K	7x1 mL
Goat anti-Mouse IgG Blue - 10 peaks, 10 ⁶ /mL	5.0-5.9	MPAK-5067-10K	10x1 mL
Goat anti-Mouse IgG Blue - Odd # peaks, 10 ⁶ /mL	5.0-5.9	MPAK-5067-5A	5x1 mL
Goat anti-Mouse IgG Blue - Even # peaks, 10 ⁶ /mL	5.0-5.9	MPAK-5067-5B	5x1 mL
Streptavidin Blue - 10 peaks, 10 ⁶ /mL	5.0-5.9	SVPAK-5067-10K	10x1 mL
Streptavidin Blue - Odd # peaks, 10 ⁶ /mL	5.0-5.9	SVPAK-5067-5A	5x1 mL
Streptavidin Blue - Even # peaks, 10 ⁶ /mL	5.0-5.9	SVPAK-5067-5B	5x1 mL

Most state-of-the-art single laser flow cytometers can resolve the 3.6 μm, 4.0 μm and 5.1 μm Blue PAKs easily in FSC/SSC channels. Ideally, one can mix them together and use both FITC and PE to run 50 assays in the same tubes with most single laser flow cytometers.

Figure 70 Histograms of Cat. No. PAK-5067-10K (Blue PAK, 5.1 μm, 10 peaks) provided by Partec GmbH.



SPHERO™ Fluorescent Particle Array Kits (PAK)

The Yellow Fluorescent Particle Kits consist of 2.8 µm and 3.6 µm particles with multiple fluorescence intensities in FITC channels. These kits are used to develop multiplex assays using either PE or PE-Cy5 tracers for detection.

The Pink Fluorescent Particle Kits consist of either 2.8 µm or 3.6 µm with twelve different intensities in the PE channel. FITC conjugates can be used for detection. They are supplied as kits of either six odd numbered peaks or six even numbered peaks for the 2.8 µm and 3.6 µm particle kits. Carboxyl Pink Fluorescent are available for covalent coupling of proteins, antibodies, or antigens. Goat anti-Mouse IgG (Fc), Streptavidin, and Biotin coated Pink Fluorescent Particles Kits are also available for other applications.

The Pink Fluorescent Particle Array Kits (FPAK) are similar to the Pink Fluorescent Particle Kits except that the FPAK particles are made from a higher quality polystyrene particle. These particles are designed for flow cytometry application. The 3.2 µm kit has nine different intensities, while the 4.3 µm kit has ten different intensities. The FPAK particles can be used in conjunction with the Blue PAKs for advanced multiplex applications.

Figure 71 SSC vs FSC histograms of Cat. Nos. FPAK-4058-10K, FPAK-3058-9K, FA-3558-6K and FB-3558-6K.

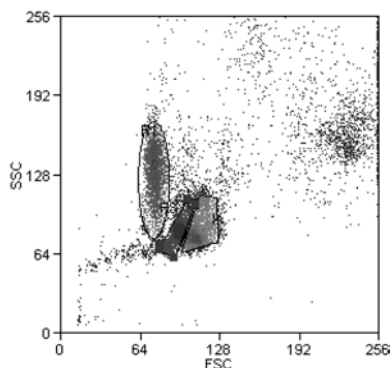
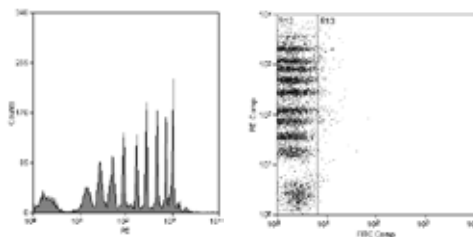


Figure 72 Histograms of Cat. No. FPAK-4058-10K (Fluorescent Particle Array Kit, Pink, 10 peaks, 4.3 µm, 1E8/mL, 10x1 mL).



Particle Type and Surface	Size, µm	Catalog No.	Unit
Yellow, Odd # peaks, 0.5% w/v	2.5-2.9	FA-2552-6K	6x1 mL
Yellow, Even # peaks, 0.5% w/v	2.5-2.9	FB-2552-6K	6x1 mL
Pink, 9 peaks, 10 ⁸ /mL	3.0-3.4	FPAK-3058-9K	9x1 mL
Pink, Odd # peaks, 0.5% w/v	3.5-3.9	FA-3558-6K	6x1 mL
Pink, Even # peaks, 0.5% w/v	3.5-3.9	FB-3558-6K	6x1 mL
Pink, 10 peaks, 10 ⁸ /mL	4.0-4.5	FPAK-4058-10K	10x1 mL
Yellow, 9 peaks, 0.5% w/v	3.5-3.9	FX-3552-9K	9x1 mL
Carboxyl Yellow, Odd # peaks, 0.25% w/v	2.5-2.9	CFA-2552-6K	6x1 mL
Carboxyl Yellow, Even # peaks, 0.25% w/v	2.5-2.9	CFB-2552-6K	6x1 mL
Carboxyl Yellow, 9 peaks, 0.25% w/v	3.5-3.9	CFX-3552-9K	9x1 mL
Carboxyl Yellow, 3 peaks, 10 ⁷ /mL	7.0-7.9	CPAK-7052-3K	3x1 mL
Carboxyl Yellow, 6 peaks, 10 ⁷ /mL	7.0-7.9	CPAK-7052-6K	6x1 mL
Carboxyl Pink, Odd # peaks, 0.25% w/v	2.5-2.9	CFA-2558-6K	6x1 mL
Carboxyl Pink, Even # peaks, 0.25% w/v	2.5-2.9	CFB-2558-6K	6x1 mL
Carboxyl Pink, Odd # peaks, 0.25% w/v	3.5-3.9	CFA-3558-6K	6x1 mL
Carboxyl Pink, Even # peaks, 0.25% w/v	3.5-3.9	CFB-3558-6K	6x1 mL
Goat anti-Mouse IgG (Fc), Pink, Odd # peaks, 0.1% w/v	3.5-3.9	MFA-3558-6K	6x1 mL
Goat anti-Mouse IgG (Fc), Pink, Even # peaks, 0.1% w/v	3.5-3.9	MFB-3558-6K	6x1 mL
Streptavidin Yellow, Odd # peaks, 0.1% w/v	2.5-2.9	SVFA-2552-6K	6x1 mL
Streptavidin Yellow, Even # peaks, 0.1% w/v	2.5-2.9	SVFB-2552-6K	6x1 mL
Streptavidin Pink, Odd # peaks, 0.1% w/v	2.5-2.9	SVFA-2558-6K	6x1 mL
Streptavidin Pink, Even # peaks, 0.1% w/v	2.5-2.9	SVFB-2558-6K	6x1 mL
Biotin Pink, Odd # peaks, 0.1% w/v	3.5-3.9	TFA-3558-6K	6x1 mL
Biotin Pink, Even # peaks, 0.1% w/v	3.5-3.9	TFB-3558-6K	6x1 mL

SPHERO™ UV Carboxyl Particle Array Kits (UVCPAK)

- Designed for multiplex flow assays on commercially available flow cytometers with UV or Violet excitation
- Detected in the UV and Violet channels
- Display minimal fluorescence in channels with 488 nm, 532 nm, and 633 nm excitation
- Available in 5.0 μm , 14 peaks at 1×10^8 particles/mL
- Carboxylated for the attachment of your specific antibodies.

Particle Type and Surface	Size, μm	Catalog No.	Unit
Carboxyl UV, 14 peaks, $10^7/\text{mL}$	5.0-5.9	UVCPAK-5042-14K	14x1 mL

The UVCPAK allows the expansion of multiplex assays with additional detection channels. Analytes of interest can be simultaneously quantified using flow cytometric analysis and any fluorescent conjugates with 488 nm, 532 nm, or 633 nm excitation.

Figure 73 Histograms of Cat. No. UVCPAK-5042-14K (UV Carboxyl Particle Array Kit, 14 peaks, $1 \times 10^8/\text{mL}$, 5.0-5.9 μm , 14x1 mL).

