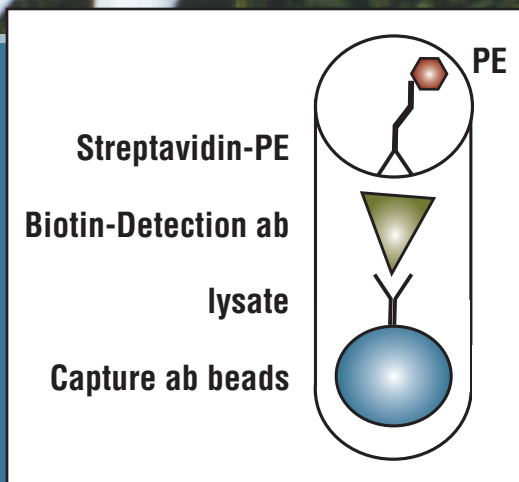




Bead Based Flow Cytometry Assays for the Analysis of Cellular Signaling

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INTRODUCTION:

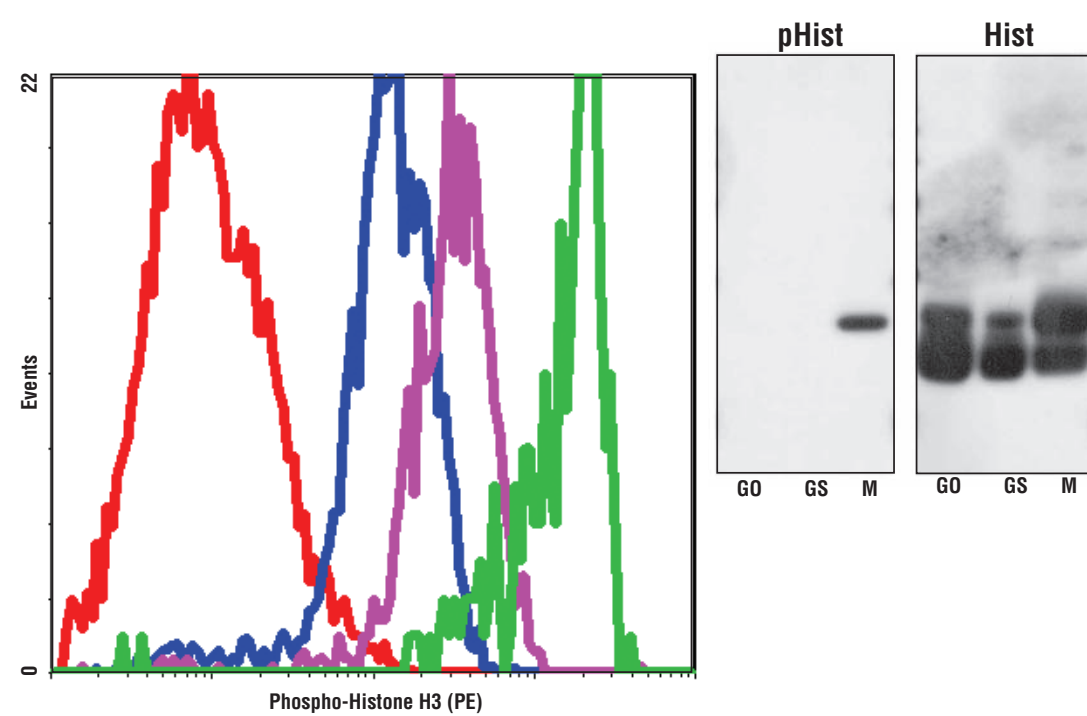
The use of antibody-labeled beads in flow cytometry allows for the multi-parametric analysis of cell and tissue lysates. The addition of activation state-specific (eg. phospho-specific) antibodies extends this method to the analysis of cell signaling in cell lines and patient samples. In this poster we describe the use of bead-based flow cytometric assays to profile the cellular signaling events associated with a variety of cancer model systems.

METHODS:

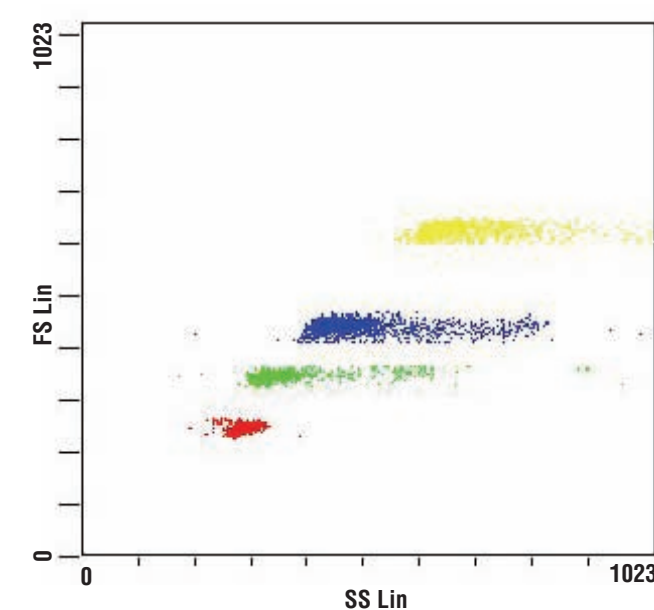
Polystyrene beads (Spherotech Inc.) were conjugated with capture antibodies and incubated with lysates from cancer cell lines treated with agents that affect the phosphorylation state of target proteins. Captured phospho proteins were labeled with biotin-conjugated phospho-sensitive detection antibodies and streptavidin-PE. The bead complexes were then analyzed on an FC500 flow cytometer (Beckman Coulter).

CONCLUSIONS:

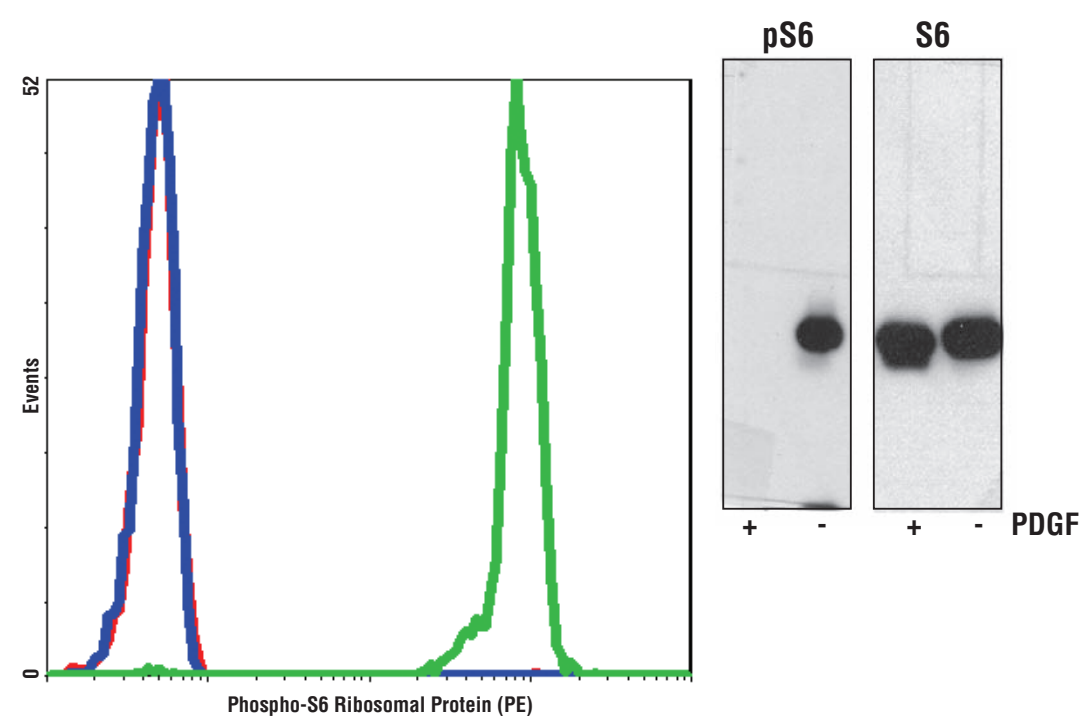
These results demonstrate that beads labeled with activation state specific antibodies can be used to perform quantifiable multi-parameter flow cytometric analyses of cellular signaling from biological samples.



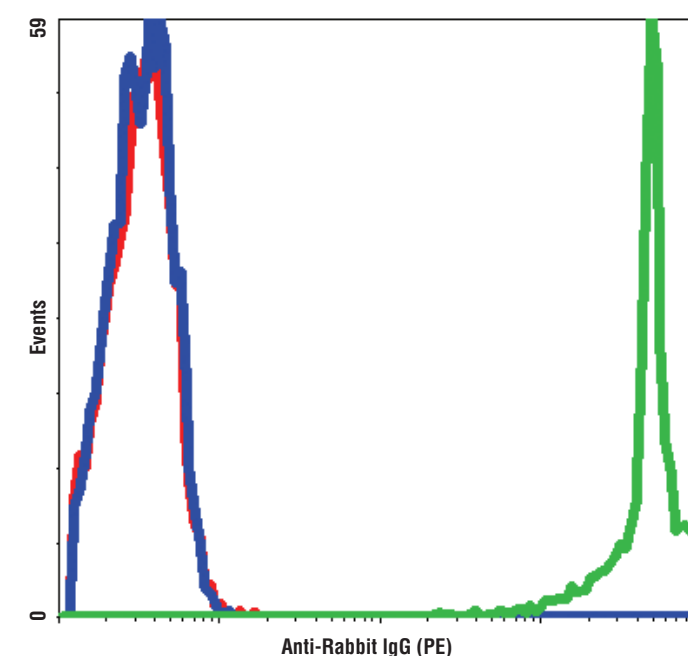
Phospho-Histone H3 (S10) bead-based analysis of lysates from HeLa cells synchronized in G0- (blue), G1/S- (magenta) or M-phase (green). Red = no detection antibody



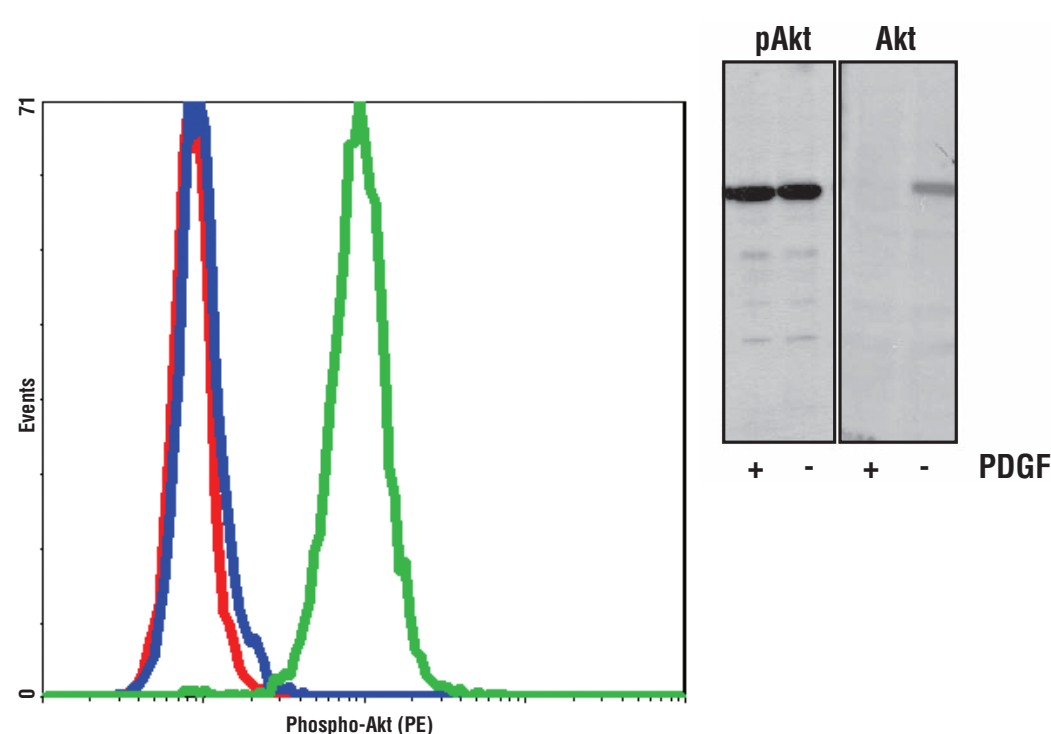
Detection of 3.2, 4.1, 5.7, and 6.7 um unconjugated polystyrene beads (red, green, blue, and yellow, respectively) by flow cytometry based on forward and side scatter.



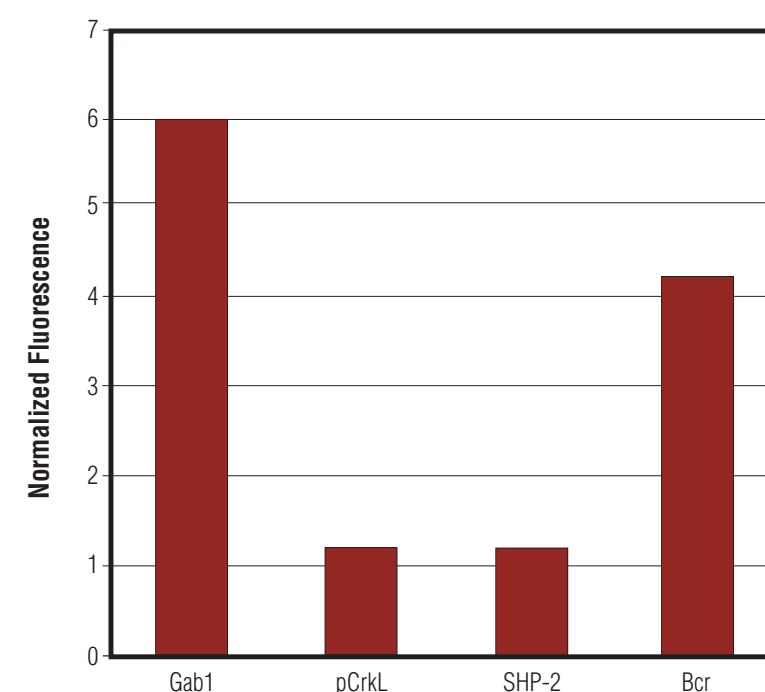
Phospho-S6 Ribosomal Protein (Ser235/236) bead-based analysis of lysates from untreated (blue) and PDGF-treated (green) NIH3T3 cells. Red = no detection antibody



Validation of S6 antibody-conjugated beads using biotinylated anti-rabbit IgG antibody and streptavidin-PE (green). Omission of anti-rabbit antibody (blue) or use of unconjugated beads (red) abolished signal.



Phospho-Akt (S473) bead-based analysis of lysates from untreated (blue) and PDGF-treated (green) NIH3T3 cells. Red = no detection antibody



BCR/ABL Bead-based analysis of lysates from K562 cells using Bcr/Abl antibody-conjugated beads. Phosphorylated Bcr and associated Gab1 protein were detected. Phospho-CrkL and SHP-2 protein were not detected, associated with active Bcr/Abl.