Bio-Plex™ suspension array system

tech note 2981

Simultaneous Detection of 12 Phosphorylated and 7 Total Proteins Using the Bio-Plex Suspension Array System

Qian Gao, Kris Simonyi, Claudia Suen, Judy Zhu-Shimoni, and Sophie Allauzen, Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547 USA

Introduction

Protein phosphorylation plays important roles in signal transduction, from activation of cell surface receptors to activation of transcription factors. To study intracellular signal transduction, it is important to detect the phosphorylation state of a target protein, which is directly related to its activity. To this end, the phosphorylation sites of many signal transduction proteins have been identified, and protein interactions have been profiled in a number of different pathways.

Phosphoprotein-specific antibodies are now available, enabling researchers to probe specific phosphorylated proteins. Traditionally, these types of investigations were carried out by western blot analysis, a lengthy process with relatively low throughput. More recently, the introduction of the Bio-Plex suspension array system, which uses an array of fluorescent microspheres and a dedicated reader with a digital processor (Figure 1), has allowed quantitation of up to 100 distinct analytes in a single sample.

We have developed a multiplex immunoassay panel that simultaneously measures the phosphorylation levels of 12 proteins (phospho-Akt, -ATF-2, -ERK, -GSK-3α/β, -IκB-α, -JNK, -p38 MAPK, -p70 S6 kinase, -p90RSK, -STAT2, -STAT3, and -TrkA) in a single well of a 96-well microplate filter. We also developed singleplex assays for phospho-EGFR and -S6. To gain a more comprehensive understanding of phosphorylation in a sample, we also developed a multiplex assay panel for simultaneous detection of 7 total target (phosphorylated and unphosphorylated) cell signaling proteins, Akt, ATF-2, ERK2, IκB-α, JNK, p38 MAPK, and p90RSK. These Bio-Plex phosphoprotein and total target assays can be used, repsectively, to measure the level of phosphorylation or total protein to generate up to 1,248 data points from a single 96-well plate.

Here we describe the performance of both types of multiplex assay panels, which have been validated with various cell lysates by correlation with western blots.

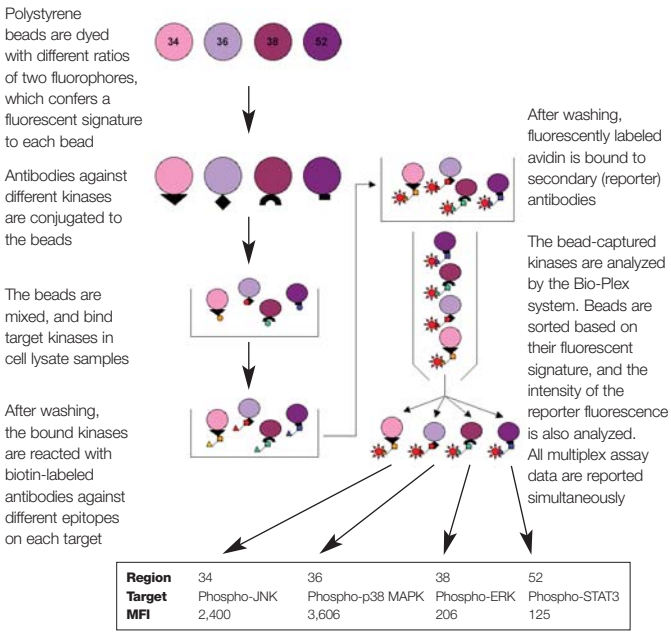

Fig. 1. Bio-Plex phosphoprotein assays.

Table 1. Target information.

Target Protein	Antibody-Reactive Species	Cell Lysate Species Tested	Phosphorylation Site(s)
Phopsho-Akt	Human, mouse, rat	Human, mouse	Ser^{473}
Phospho-ATF-2	Human, mouse, rat	Human, mouse	Thr^{71}
Phospho-EGFR*	Human	Human, mouse	Tyr
Phospho-ERK1/2	Human, mouse, rat	Human, mouse	Thr^{202}/Tyr^{204}, Thr^{185}/Tyr^{187}
Phospho-GSK-3α/β	Human, mouse, rat	Human, mouse	Ser^{21}/Ser^{9}
Phospho-IκB-α	Human, mouse	Human, mouse	Ser^{32}/Ser^{36}
Phospho-JNK	Human, mouse	Human, mouse	Thr^{183}/Tyr^{185}
Phospho-p38 MAPK	Human, mouse, rat	Human, mouse	Thr^{180}/Tyr^{182}
Phospho-p70 S6 kinase	Human, mouse, rat	Human, mouse	Thr^{421}/Ser^{424}
Phospho-p90RSK	Human, mouse, rat	Human, mouse	Thr^{359}/Ser^{363}
Phospho-S6*	Human, mouse, rat	Human, mouse	Ser^{235}/Ser^{236}
Phospho-STAT2	Human	Human	Tyr^{689}
Phospho-STAT3	Human, mouse, rat	Human, mouse	Tyr^{705}
Phospho-TrkA	Human, mouse, rat	Human, rat	Tyr^{496}

* Bio-Plex singleplex assay

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Methods

Treated and untreated control cultures were prepared at the same time. Cultured cells were lysed according to the instructions specified in the Bio-Plex cell lysis kit product insert. An equal volume of assay buffer from the Bio-Plex phosphoprotein reagent kit was added to each sample. The cell culture lysates were analyzed according to the instructions specified in the Bio-Plex phosphoprotein detection instruction manual. The protocols for lysate preparation and assays are summarized in Figure 2.

Cell Lysate Preparation	Assay Procedures
Treat cells as desired	Prewet filter plate
Wash	Add antibody-conjugated beads
Add cell lysis buffer	**Wash**
Scrape and collect to a centrifuge tube	Incubate with cell lysate in assay buffer
Centrifuge	**Wash**
Collect supernatants	Incubate with detection antibodies
	Wash
	Incubate with streptavidin-PE
	Wash
	Resuspend beads
	Read on Bio-Plex suspension array system

Fig. 2. Protocols for lysate preparation and assays.

Results and Discussion

Table 1 identifies the target proteins investigated here and their respective phosphorylation sites. Bio-Plex assays were tested for correlation with western blots for 14 phosphoprotein targets using lysates from different treated cell lines. The cell lysates were diluted, and for each dilution, the same amount of total protein (µg per well or lane) was tested both by Bio-Plex assay and western blotting. Figure 3 summarizes the results. A good dose response was demonstrated for each of the 14 diluted targets, and the mean fluorescence intensity (MFI) from the Bio-Plex phosphoprotein assays correlated very well with the band intensities seen in western blots.

Bio-Plex total target assays were also compared to western blots for serially diluted untreated HEK 293, HeLa, or NIH 3T3 cell lysates. For each dilution, the same amount of total protein (µg per well or lane) was tested both by Bio-Plex assay and western blotting. Figure 4 shows the correlation between the Bio-Plex total target assay MFI values and intensity of the bands on the western blots. All 7 of the Bio-Plex total target assays demonstrated a linear dose-response and good correlation with western blots.

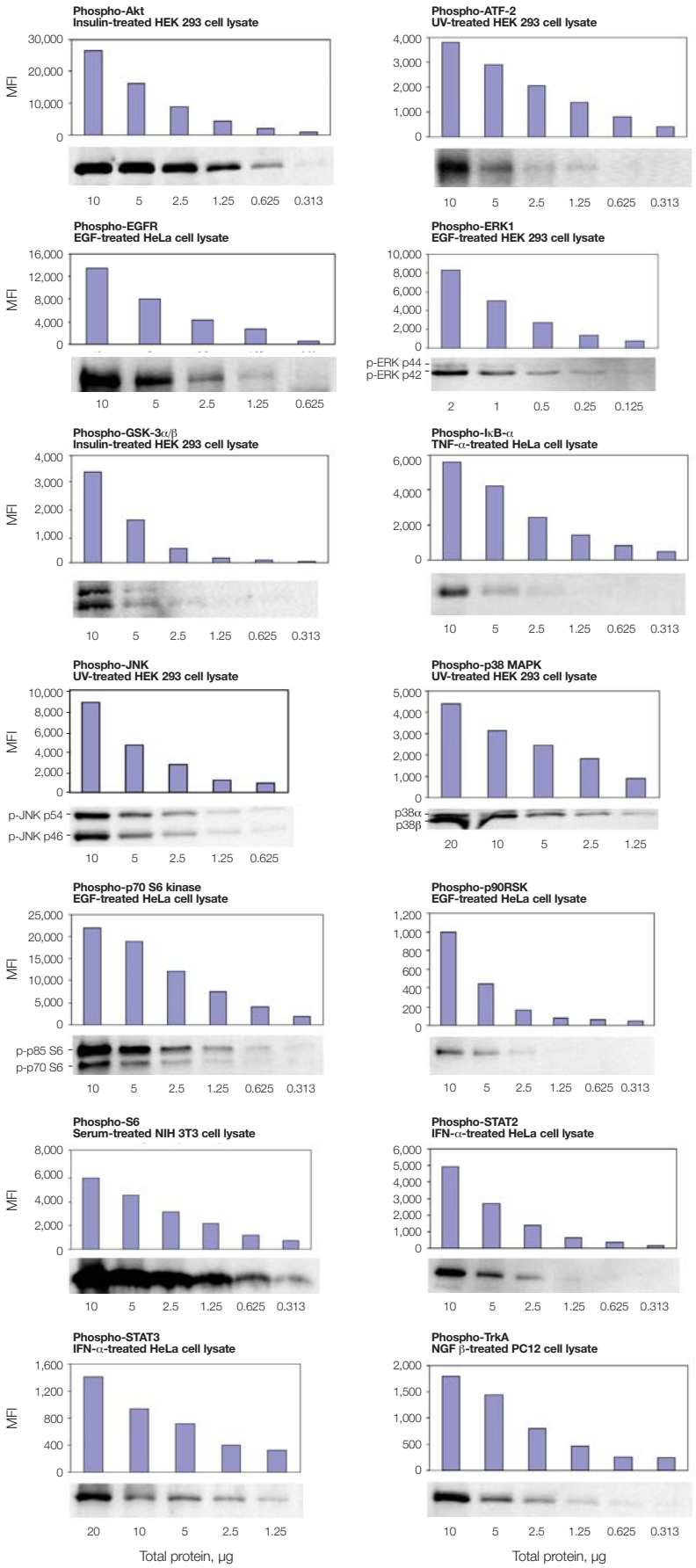

Fig. 3. Correlation of Bio-Plex phosphoprotein assays (upper panels) with western blots (lower panels). Note correlation of assay MFI values to band intensities. To compensate for the low-level phosphorylation of phospho-ATF-2 in cultured cells, mouse tissue expressing a high level of ATF-2 was used.

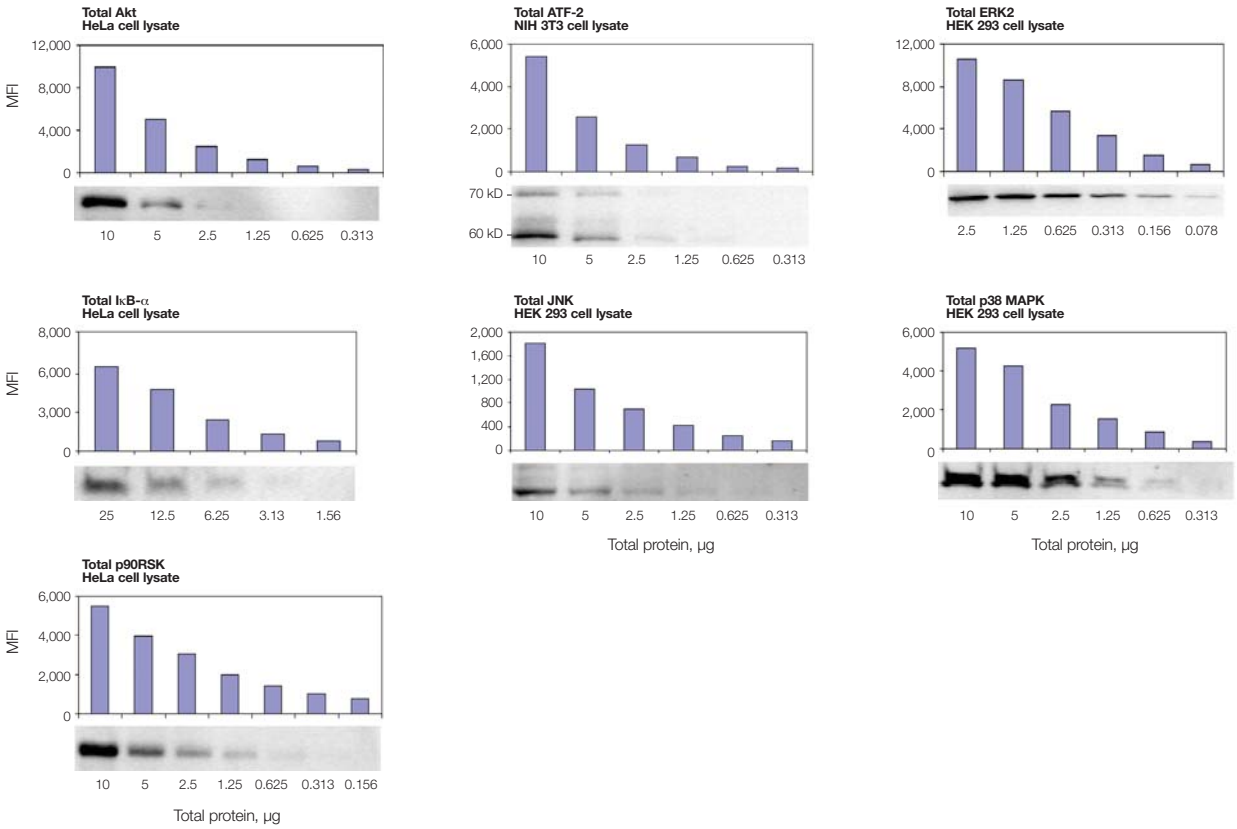

Fig. 4. Correlation of Bio-Plex total target assays (upper panels) with western blots (lower panels). Note correlation of assay MFI value to band intensities.

Conclusions

- The multiplex Bio-Plex phosphoprotein or total assays detect different phosphorylated or total targets simultaneously from one well of a 96-well plate
- Currently, Bio-Plex assays are available for 12 multiplexable phosphoprotein and 7 multiplexable total assays
- Bio-Plex singleplex assays for phospho-EGFR and phospho-S6 have also been developed
- These multiplex phosphoprotein assay panels exhibit greater or equal sensitivity compared to western blots

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