

# Simultaneous Detection of Multiple Phosphorylated and Total Proteins Using the Bio-Plex Suspension Array System

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## Abstract

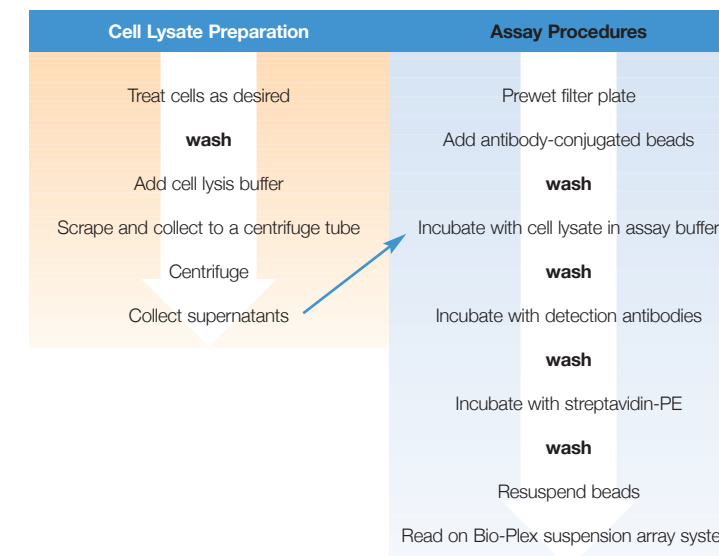
Dysfunctions in kinase activity (resulting from environmental factors, genetic abnormalities, or viral infection) are associated with stress, diseases such as cancer, and apoptosis. The ability to simultaneously measure phosphorylation of specific proteins involved in signal transduction pathways has become more significant in several disease research areas. The Bio-Plex suspension array system uses an array of fluorescent microspheres and a dedicated reader with a high-speed digital processor. This technology has made it possible to quantitate up to 100 distinct analytes in one sample. Commercially available multiplexable phosphoprotein assays from Bio-Rad Laboratories include 19 phosphorylated targets and 8 total protein targets. To further expand our selection, we have developed 6 new phosphoprotein targets (p-IRS-1, p-STAT6, p-PDGFR, p-CREB, p-histone H3 and p-MEK1) and 4 new total protein targets (t-ERK1/2, t-HSP27, t-CREB, and t-MEK1). We are also presenting preliminary data from new assays under consideration for the Bio-Plex format. The performance of both phosphoprotein and total target panels was validated with various cell lysates in terms of correlation with western blots, reproducibility, and specificity. These highly multiplex assay panels are powerful tools for the investigation of cell signaling pathways and inhibitor screening for new drug discovery.

## Target Protein Phosphorylation Sites

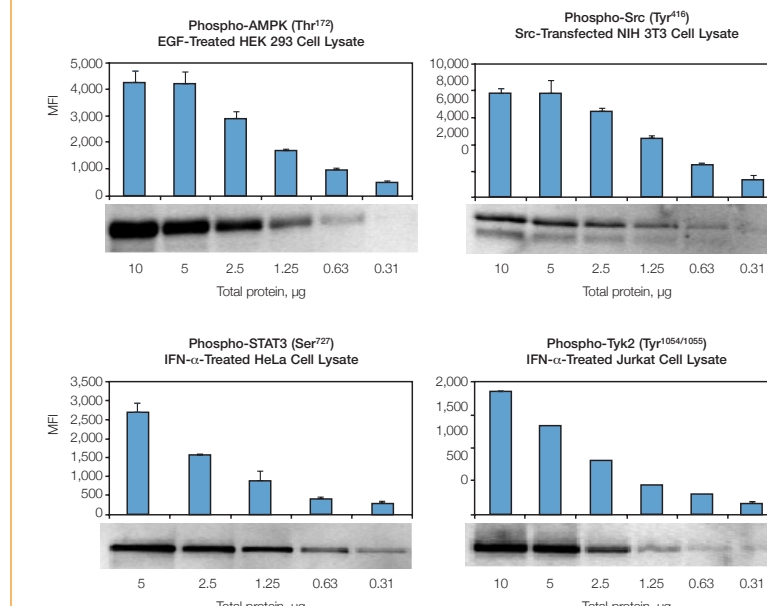
Assays	Targeted Analytes	Antibody Reactive Species	Phosphorylation Site(s)
Available	Phospho-Akt	Human, mouse	Ser <sup>473</sup>
	Phospho-ATF-2	Human, mouse	Tyr <sup>71</sup>
	Phospho-c-Jun	Human, mouse, rat	Ser <sup>83</sup>
	Phospho-EGFR*	Human, mouse	p-Tyr
	Phospho-ERK1/2	Human, mouse	Thr <sup>202</sup> , Tyr <sup>204</sup> /Thr <sup>183</sup> , Tyr <sup>185</sup>
	Phospho-GSK-3 $\alpha/\beta$	Human, mouse	Ser <sup>21/9</sup>
	Phospho-HSP27	Human, monkey	Ser <sup>78</sup>
	Phospho-I $\kappa$ B $\alpha$	Human, mouse	Ser <sup>30/36</sup>
	Phospho-JNK1/2	Human, mouse	Thr <sup>183</sup> , Tyr <sup>185</sup>
	Phospho-NF- $\kappa$ B p65	Human	Ser <sup>536</sup>
	Phospho-p38 MAPK $\alpha/\beta$	Human, mouse	Thr <sup>180</sup> , Tyr <sup>182</sup>
	Phospho-p53	Human, mouse	Ser <sup>15</sup>
	Phospho-p70 S6 kinase	Human, mouse	Thr <sup>421</sup> , Ser <sup>424</sup>
	Phospho-p90RSK	Human, mouse	Thr <sup>359</sup> , Ser <sup>363</sup>
	Phospho-S6*	Human, mouse	Ser <sup>235/236</sup>
	Phospho-STAT2	Human	Tyr <sup>689</sup>
	Phospho-STAT3	Human, mouse	Tyr <sup>703</sup>
	Phospho-TrkA	Human, rat	Tyr <sup>496</sup>
	Coming Soon	Phospho-Akt	Human, rat
Phospho-CREB		Human, mouse, rat	Ser <sup>133</sup>
Phospho-histone H3		Human, mouse, rat, monkey	Ser <sup>10</sup>
Phospho-IRS-1		Human, mouse, rat	Ser <sup>636/639</sup>
Phospho-MEK1		Human, mouse, rat	Ser <sup>217/221</sup>
Phospho-PDGFR		Human, mouse, rat	Tyr <sup>751</sup>
Phospho-STAT6		Human, mouse	Tyr <sup>641</sup>

\* This assay is not multiplexable.

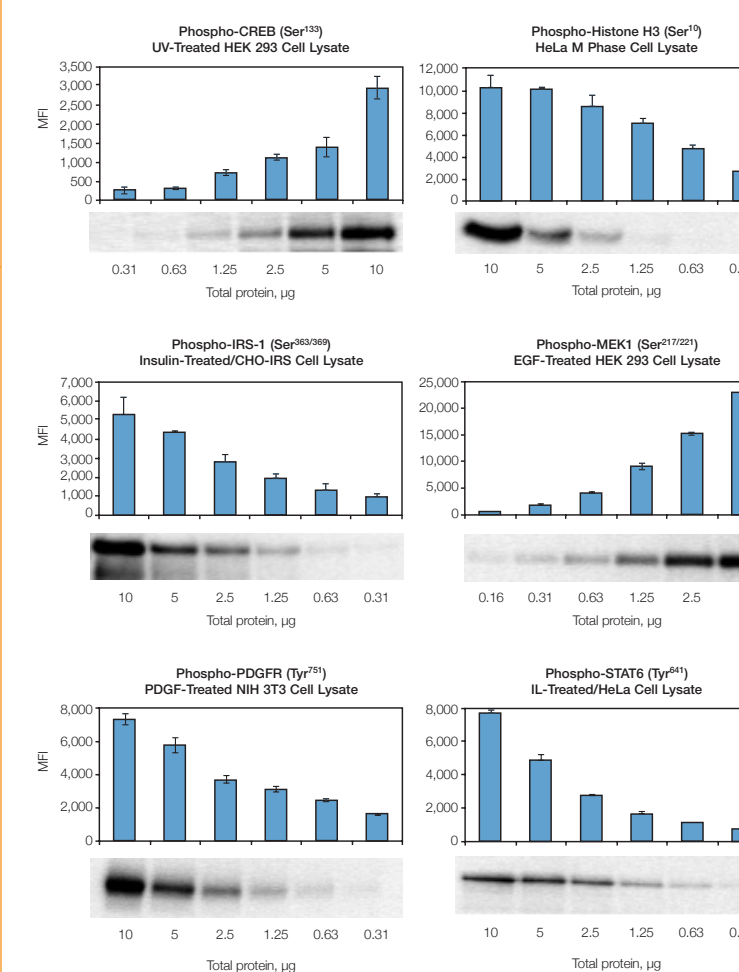
## Experimental Procedures



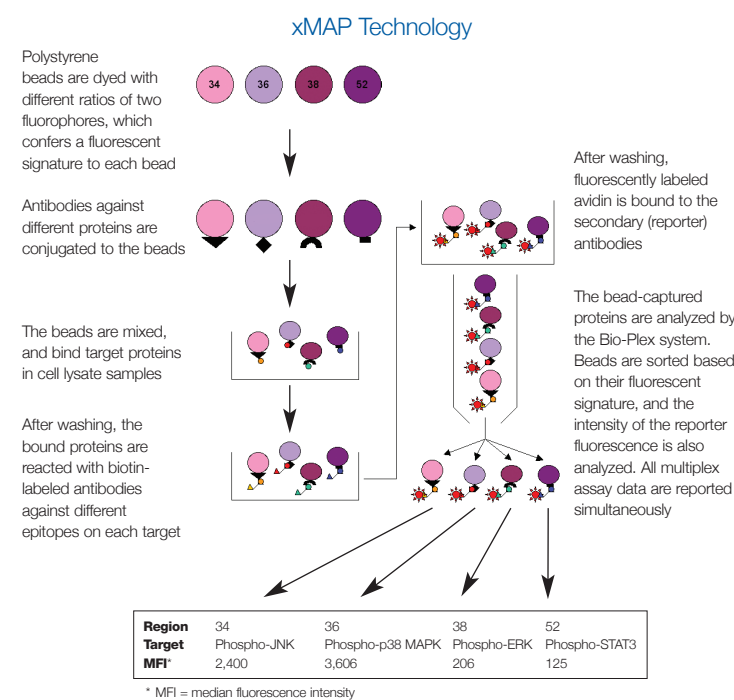
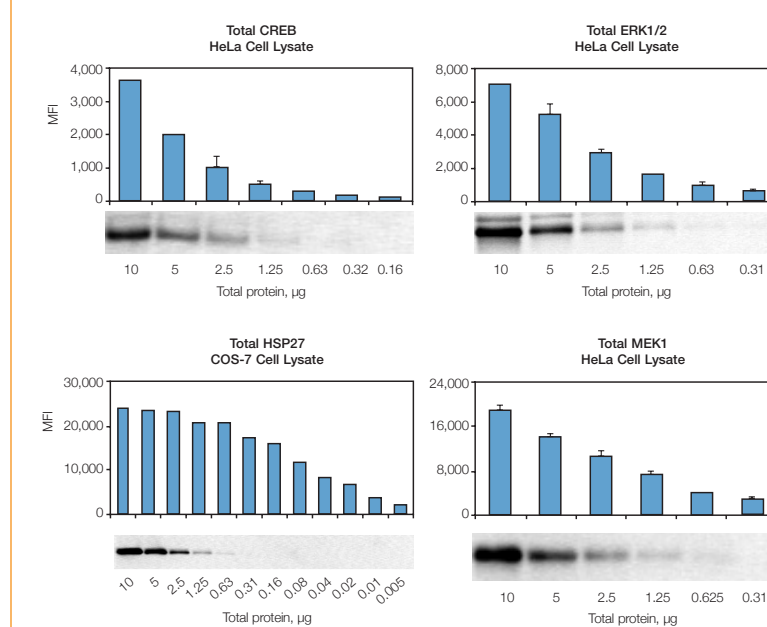
## Research Stage Bio-Plex Phosphoprotein Assay Correlation With Western Blots



## Newly Developed Bio-Plex Phosphoprotein Assay Correlation With Western Blots

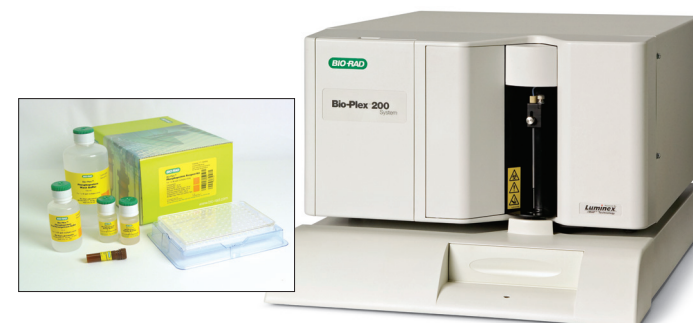


## Newly Developed Bio-Plex Total Kinase Assay Correlation With Western Blots



Region	34	36	38	52
Target	Phospho-JNK	Phospho-p38 MAPK	Phospho-ERK	Phospho-STAT3
MFI	2,400	3,606	206	125

\* MFI = median fluorescence intensity



## Total Target Assay Targets

Assays	Targeted Analytes	Antibody Reactive Species
Available	Akt	Human, mouse
	ATF-2	Human, mouse
	c-Jun	Human, mouse, rat
	ERK2	Human, mouse
	I $\kappa$ B- $\alpha$	Human, mouse
	JNK 1/2	Human, mouse
	p38 MAPK	Human, mouse
	p90RSK	Human, mouse
Coming Soon	CREB	Human, mouse, rat
	ERK1/2	Human, mouse
	HSP27	Human
	MEK1	Human, mouse, rat

## Conclusions

- The multiplex Bio-Plex total or phosphoprotein assays allow detection of different phosphorylated targets simultaneously from one well of a 96-well plate.
- We will soon offer a larger selection of 23 multiplexable phosphoprotein and 12 multiplexable total assays.
- Bio-Plex singleplex assays for p-EGFR and p-S6 have also been developed.
- These multiplex phosphoprotein assay panels exhibit greater or equal sensitivity compared to western blots.

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