

## Bio-Plex Phosphoprotein Detection Performance

### Introduction

The role of protein kinases in regulating cell behavior has led to their investigation as targets for treatment of a variety of diseases, including cancer, diabetes, osteoporosis, inflammation, and ocular diseases. Bio-Plex phosphoprotein and total target assays are multiplex bead-based assays (using xMAP technology) that detect the phosphorylation and expression of proteins in lysates derived from cell culture or tissue samples. The Bio-Plex total target assay reports the abundance of the target protein in one well, while the Bio-Plex phosphoprotein assay reports the phosphorylation level of the same protein in a separate well. This technical information sheet illustrates the performance of Bio-Plex phosphoprotein and total target assays. All targets have been validated with various cell lysates by correlation with western blots.

### Methods

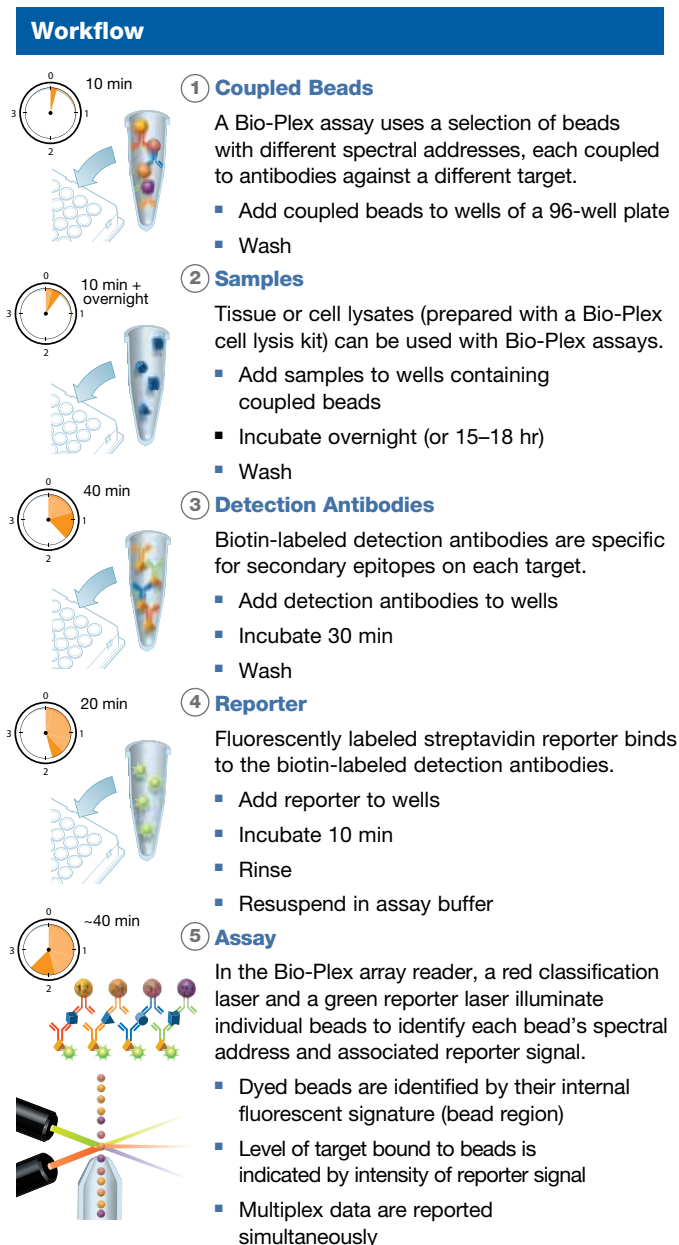
#### Instruments and Reagents

Bio-Plex phosphoprotein detection reagent kits must be used with phosphoprotein or total target assays, either in a singleplex assay or an x-Plex™ multiplex assay format. To obtain optimal recovery, cell culture or tissue samples must be prepared using the Bio-Plex cell lysis kit. The following instruments and reagents were used to generate the data in this document:

- Available Bio-Plex phosphoprotein and total target assays
- Bio-Plex cell lysis kit
- Bio-Plex phosphoprotein detection reagent kit
- Bio-Plex validation kit
- Bio-Plex calibration kit
- Bio-Plex Manager™ software, version 4.1
- Bio-Plex suspension array system

#### Protocol

Assays were performed according to the workflow in Figure 1. Refer to the Bio-Plex phosphoprotein detection instruction manual for the detailed protocol. Western blot analysis was performed according to routine procedures, and the blots were probed with corresponding phosphospecific antibodies.



**Fig. 1. Bio-Plex phosphoprotein and total target assay workflow.** Spheres, beads (numbers indicate bead regions); blue symbols, phosphoproteins; green sunbursts, fluorescent label.

# Bio-Plex Phosphoprotein and Total Target Assays

## Available Assays\*

Assays	Phosphoprotein	Total
<b>Akt Signaling</b>		
Akt (Ser <sup>473</sup> )	●	●
GSK-3 $\alpha$ / $\beta$ (Ser <sup>21</sup> /Ser <sup>9</sup> )	●	
<b>Cell Cycle/Checkpoint Control</b>		
p53 (Ser <sup>15</sup> )	●	●
p53 (Ser <sup>37</sup> )	●	●
p53 (Ser <sup>46</sup> )	●	●
<b>Chromatin Regulation/Acetylation</b>		
Histone H3 (Ser <sup>10</sup> )	●	
<b>Immunology/Inflammation</b>		
I $\kappa$ B- $\alpha$ (Ser <sup>32</sup> /Ser <sup>36</sup> )	●	●
NF- $\kappa$ B p65 (Ser <sup>536</sup> )	●	
STAT2 (Tyr <sup>689</sup> )	●	
STAT3 (Ser <sup>727</sup> )	●	
STAT3 (Tyr <sup>705</sup> )	●	
STAT6 (Tyr <sup>641</sup> )	●	
Tyk2 (Tyr <sup>1054</sup> /Tyr <sup>1055</sup> )	●	
<b>Glucose/Energy Metabolism</b>		
IGF-IR (Tyr <sup>1131</sup> )	●	
IR- $\beta$ (Tyr <sup>1146</sup> )	●	
IRS-1 (Ser <sup>636</sup> /Ser <sup>639</sup> )	●	
<b>MAP Kinase Signaling</b>		
ATF-2 (Thr <sup>71</sup> )	●	●
c-Jun (Ser <sup>63</sup> )	●	●
ERK1 (Thr <sup>202</sup> /Tyr <sup>204</sup> )	●	
ERK2 (Thr <sup>185</sup> /Tyr <sup>187</sup> )	●	●
ERK1/2 (Thr <sup>202</sup> /Tyr <sup>204</sup> , Thr <sup>185</sup> /Tyr <sup>187</sup> )	●	●
HSP27 (Ser <sup>79</sup> )	●	●
JNK (Thr <sup>183</sup> /Tyr <sup>185</sup> )	●	●
MEK1 (Ser <sup>217</sup> /Ser <sup>221</sup> )	●	●
p38 MAPK (Thr <sup>180</sup> /Tyr <sup>182</sup> )	●	●
p90RSK (Thr <sup>359</sup> /Ser <sup>363</sup> )	●	●
<b>Neuroscience</b>		
CREB (Ser <sup>133</sup> )	●	●
TrkA (Tyr <sup>490</sup> )	●	
<b>Translational Control</b>		
p70 S6 kinase (Thr <sup>421</sup> /Ser <sup>424</sup> )	●	
S6 ribosomal protein (Ser <sup>235</sup> /Ser <sup>236</sup> )	●	
<b>Tyrosine Kinases</b>		
Bcr-Abl (Tyr <sup>245</sup> )	●	●
c-Abl (Tyr <sup>245</sup> )	●	
c-Abl (Tyr <sup>412</sup> )	●	
EGFR (Tyr)	●	
PDGF receptor- $\beta$ (Tyr <sup>751</sup> )	●	
Src (Tyr <sup>416</sup> )	●	

\* You can design an x-Plex assay for combinations of assays. For more information, go to [www.bio-rad.com/bio-plex/x-plex/](http://www.bio-rad.com/bio-plex/x-plex/) or contact your local Bio-Rad sales representative.

## Performance

The assay matrices identify each target protein and its multiplexability. Figures 2 and 3 (following pages) summarize representative data obtained from Bio-Plex assays and western blotting. These data show that Bio-Plex assays effectively measure phosphoprotein levels and correlate well with traditional western blotting.

## Applications

For a list of publications using Bio-Plex phosphoprotein and total target assays, refer to bulletin 5394.

## Performance Characteristics

### Specifications\*

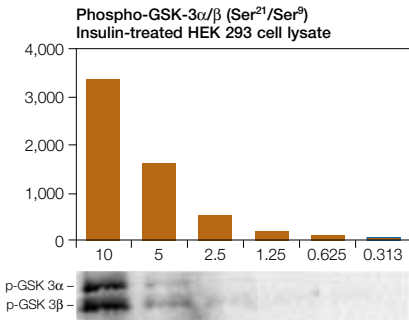
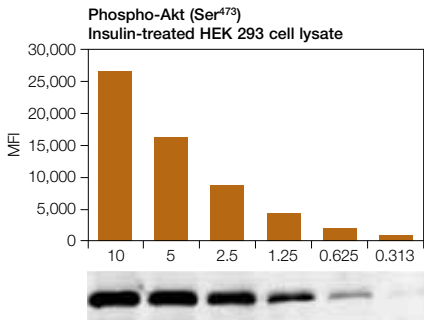
Reactive species	Human, monkey, mouse, rat
Matrices	Cell culture lysate, tissue lysate
Limit of detection	<10 $\mu$ g/ml of total protein
Precision	Intra-assay %CV <15% Inter-assay %CV <25%

\* Values vary by assay.

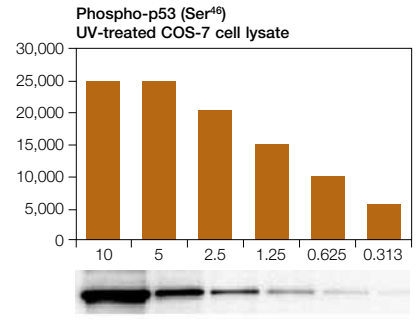
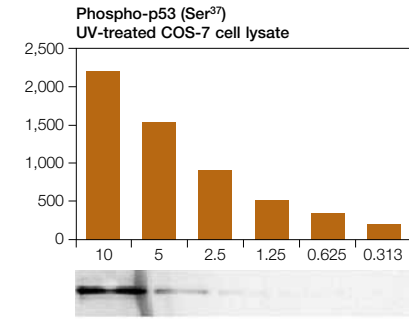
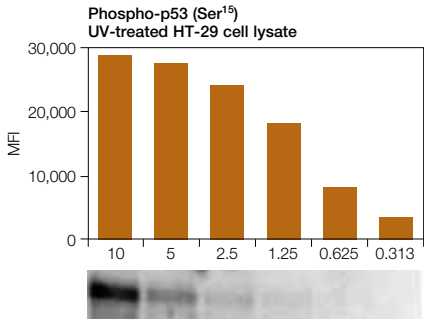




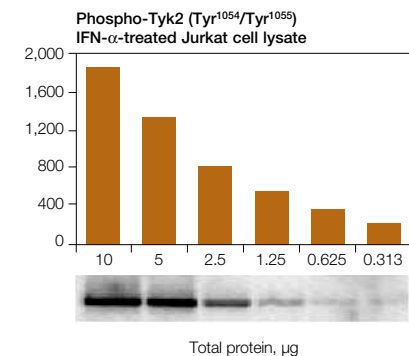
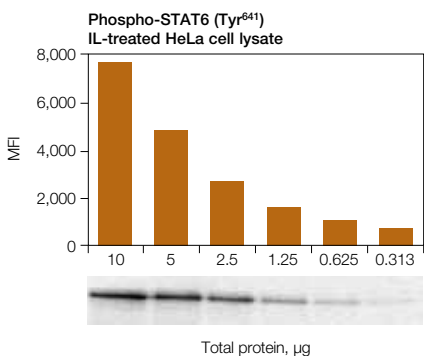
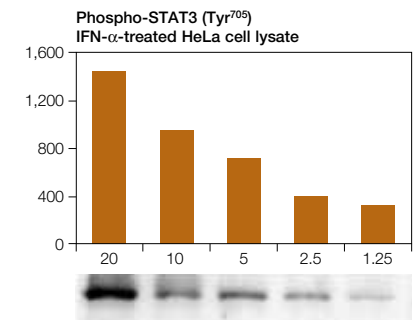
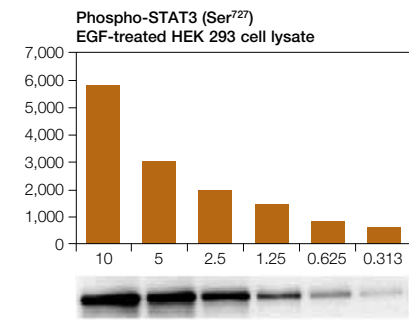
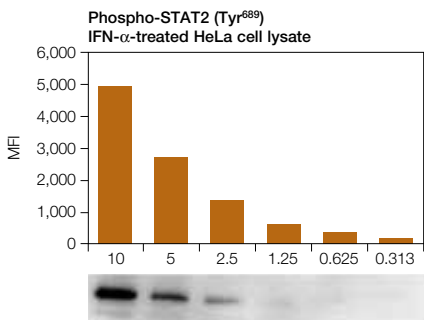
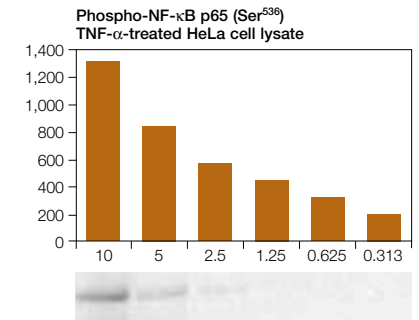
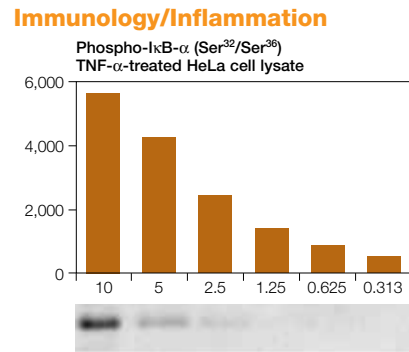
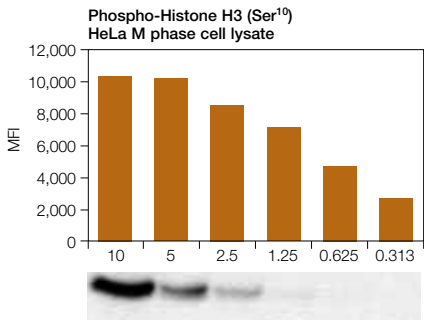
**Akt Signaling**



**Cell Cycle/Checkpoint Control**

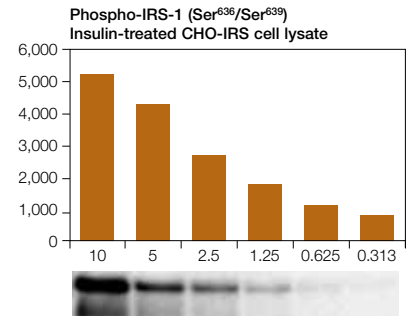
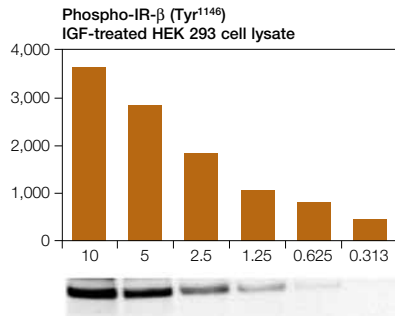
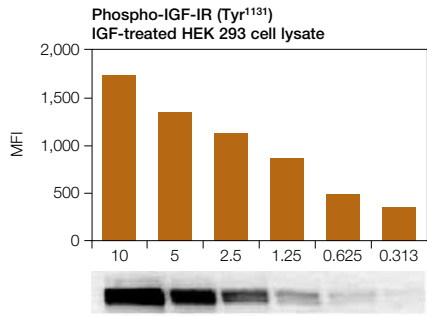


**Chromatin Regulation/Acetylation**

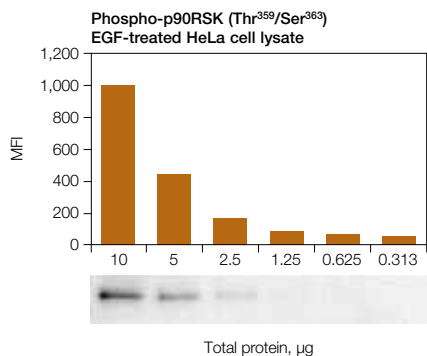
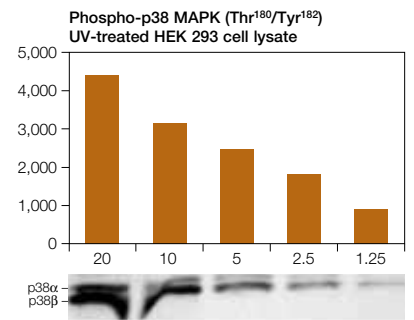
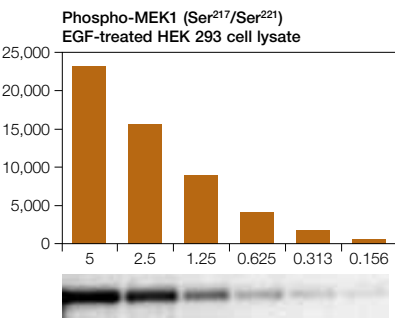
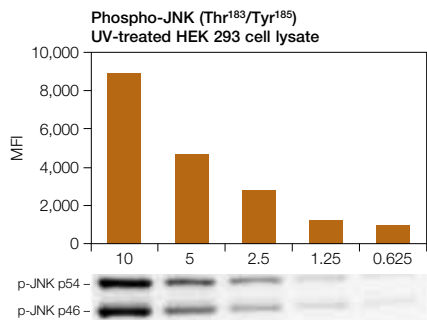
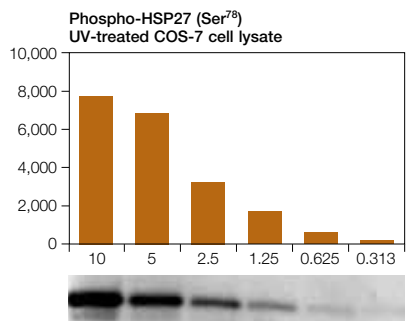
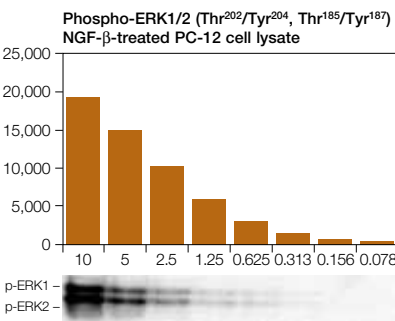
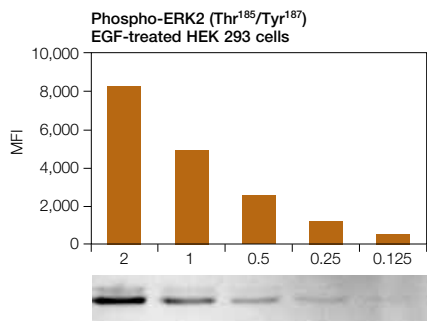
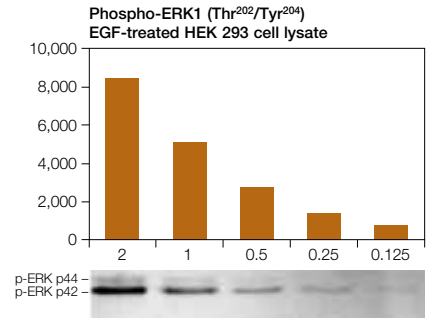
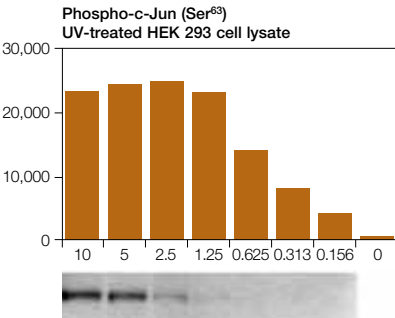
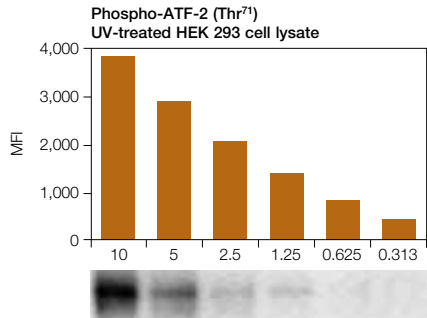


Total protein,  $\mu$ g

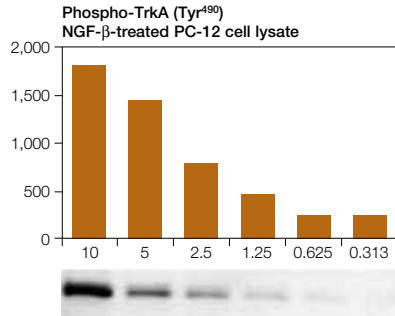
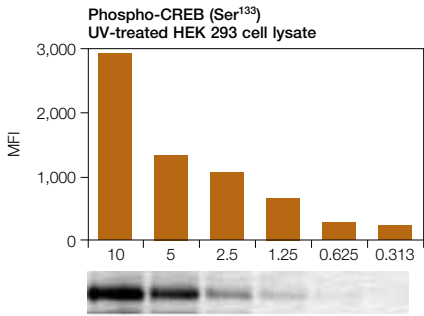
## Glucose/Energy Metabolism



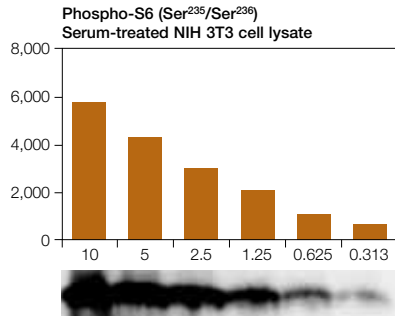
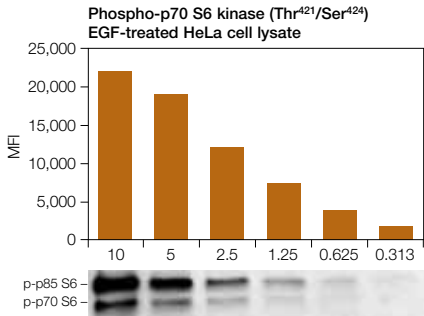
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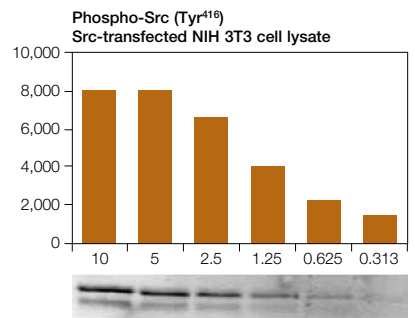
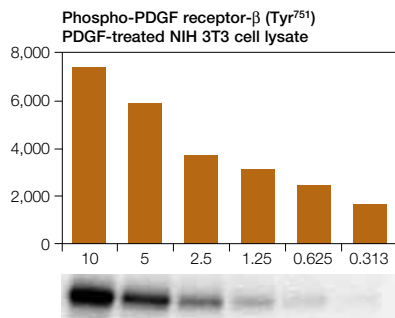
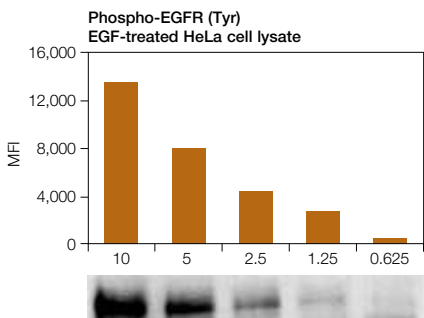
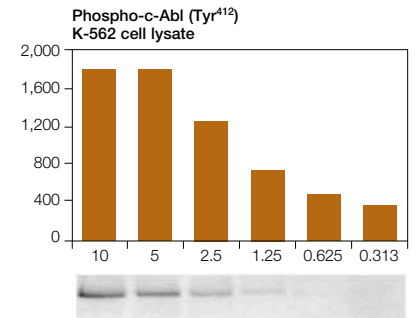
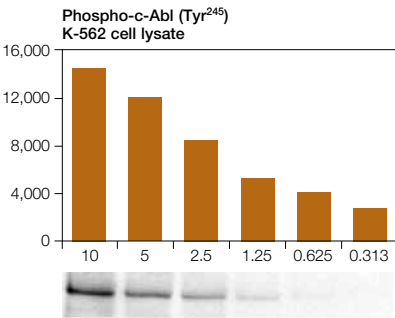
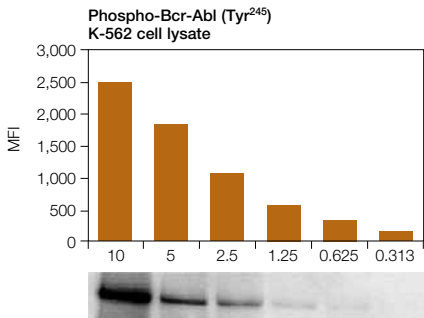
## Neuroscience



## Translational Control

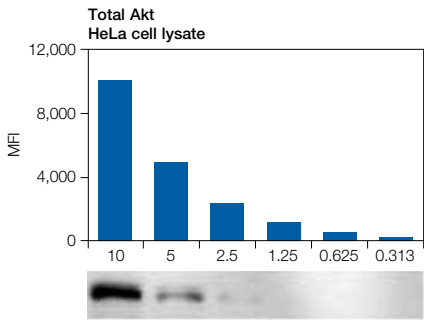


## Tyrosine Kinases

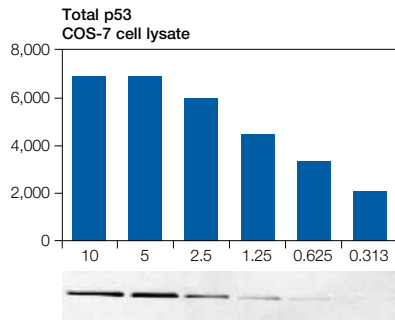


**Fig. 2. Correlation of Bio-Plex phosphoprotein assays (upper panels) with western blots (lower panels).** Note that the correlation of assay median fluorescence intensity (MFI) values to band intensities for diluted samples is total protein ( $\mu\text{g}/\text{well}$  and  $\mu\text{g}/\text{lane}$ ).

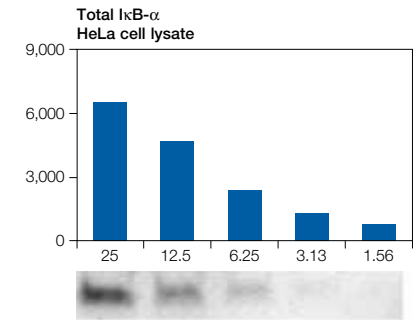
**Akt Signaling**



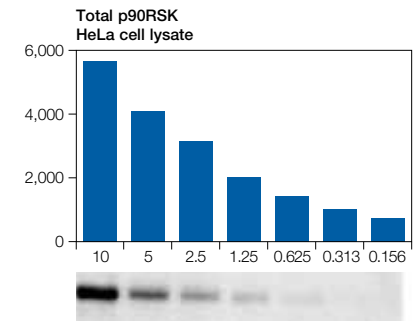
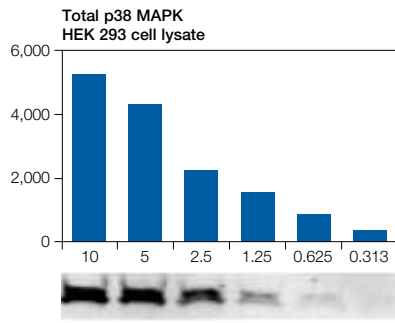
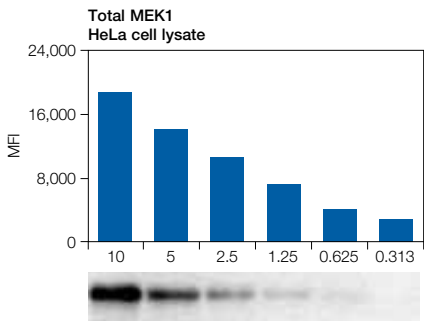
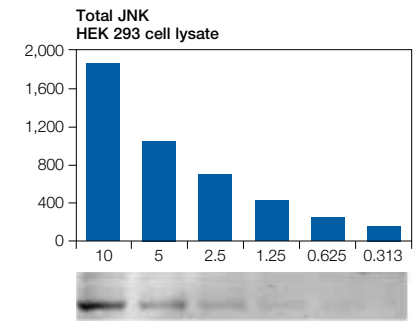
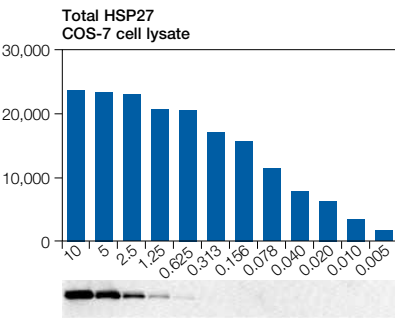
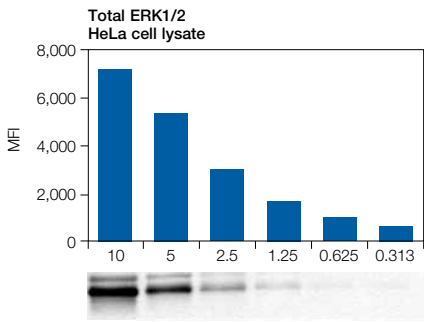
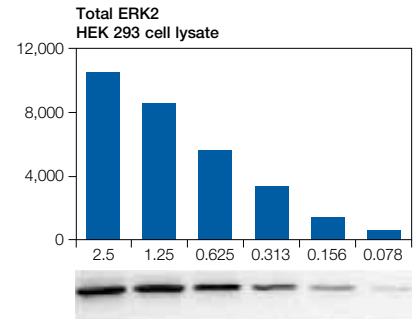
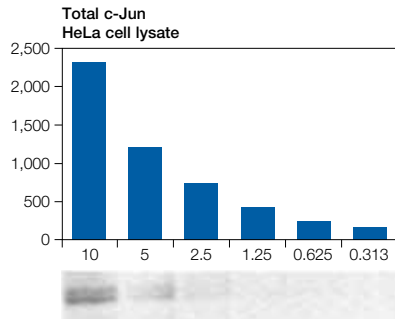
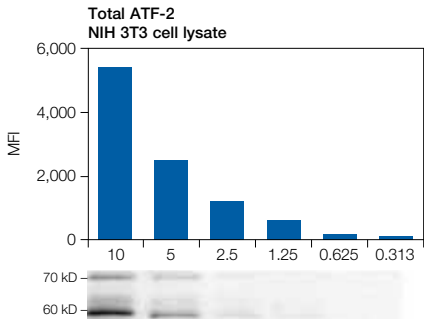
**Cell Cycle/Checkpoint Control**



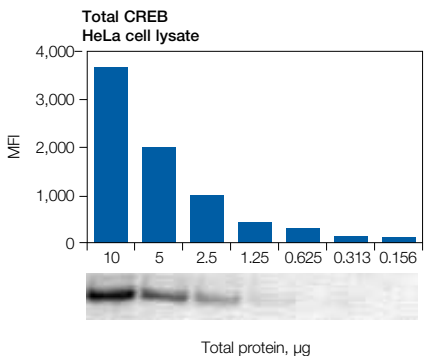
**Immunology/Inflammation**



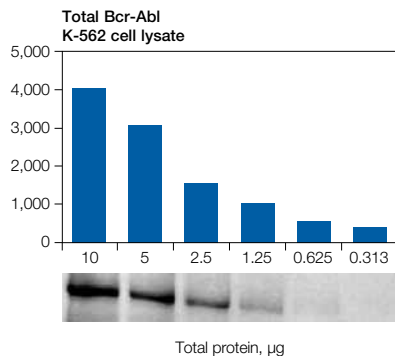
**MAP Kinase Signaling**



**Neuroscience**



**Tyrosine Kinases**



Total protein, µg

**Fig. 3. Correlation of Bio-Plex total target assays (upper panels) with western blots (lower panels).** Note that the correlation of assay median fluorescence intensity (MFI) values to band intensities for diluted samples is total protein (µg/well and µg/lane).

## Ordering Information

Catalog # Description

### Bio-Plex Phosphoprotein Singleplex Assays\*

171-V21075	Bio-Plex Phospho-Akt (Ser <sup>473</sup> ) Assay, 1 x 96-well
171-V21620	Bio-Plex Phospho-ATF-2 (Thr <sup>71</sup> ) Assay, 1 x 96-well
171-V27145	Bio-Plex Phospho-Bcr-Abl (Tyr <sup>245</sup> ) Assay, 1 x 96-well
171-V27545	Bio-Plex Phospho-c-Abl (Tyr <sup>245</sup> ) Assay, 1 x 96-well
171-V27745	Bio-Plex Phospho-c-Abl (Tyr <sup>412</sup> ) Assay, 1 x 96-well
171-V24356	Bio-Plex Phospho-c-Jun (Ser <sup>63</sup> ) Assay, 1 x 96-well
171-V26119	Bio-Plex Phospho-CREB (Ser <sup>133</sup> ) Assay, 1 x 96-well
171-V23120**	Bio-Plex Phospho-EGFR (Tyr) Assay, 1 x 96-well
171-V21938	Bio-Plex Phospho-ERK1 (Thr <sup>202</sup> /Tyr <sup>204</sup> ) Assay, 1 x 96-well
171-V20438	Bio-Plex Phospho-ERK2 (Thr <sup>185</sup> /Tyr <sup>187</sup> ) Assay, 1 x 96-well
171-V22238	Bio-Plex Phospho-ERK1/2 (Thr <sup>202</sup> /Tyr <sup>204</sup> , Thr <sup>185</sup> /Tyr <sup>187</sup> ) Assay, 1 x 96-well
171-V23318	Bio-Plex Phospho-GSK-3 $\alpha$ / $\beta$ (Ser <sup>21</sup> /Ser <sup>9</sup> ) Assay, 1 x 96-well
171-V24777	Bio-Plex Phospho-Histone H3 (Ser <sup>10</sup> ) Assay, 1 x 96-well
171-V24551	Bio-Plex Phospho-HSP27 (Ser <sup>78</sup> ) Assay, 1 x 96-well
171-V27343	Bio-Plex Phospho-IGF-IR (Tyr <sup>131</sup> ) Assay, 1 x 96-well
171-V20758	Bio-Plex Phospho-I $\kappa$ B- $\alpha$ (Ser <sup>32</sup> /Ser <sup>36</sup> ) Assay, 1 x 96-well
171-V27943	Bio-Plex Phospho-IR- $\beta$ (Tyr <sup>1146</sup> ) Assay, 1 x 96-well
171-V25576	Bio-Plex Phospho-IRS-1 (Ser <sup>636</sup> /Ser <sup>639</sup> ) Assay, 1 x 96-well
171-V21034	Bio-Plex Phospho-JNK (Thr <sup>183</sup> /Tyr <sup>185</sup> ) Assay, 1 x 96-well
171-V25340	Bio-Plex Phospho-MEK1 (Ser <sup>217</sup> /Ser <sup>221</sup> ) Assay, 1 x 96-well
171-V24937	Bio-Plex Phospho-NF- $\kappa$ B p65 (Ser <sup>536</sup> ) Assay, 1 x 96-well
171-V21336	Bio-Plex Phospho-p38 MAPK (Thr <sup>180</sup> /Tyr <sup>182</sup> ) Assay, 1 x 96-well
171-V25153	Bio-Plex Phospho-p53 (Ser <sup>15</sup> ) Assay, 1 x 96-well
171-V28353	Bio-Plex Phospho-p53 (Ser <sup>37</sup> ) Assay, 1 x 96-well
171-V28153	Bio-Plex Phospho-p53 (Ser <sup>46</sup> ) Assay, 1 x 96-well
171-V24155	Bio-Plex Phospho-p70 S6 Kinase (Thr <sup>421</sup> /Ser <sup>424</sup> ) Assay, 1 x 96-well
171-V23535	Bio-Plex Phospho-p90RSK (Thr <sup>359</sup> /Ser <sup>363</sup> ) Assay, 1 x 96-well
171-V25957	Bio-Plex Phospho-PDGF Receptor- $\beta$ (Tyr <sup>751</sup> ) Assay, 1 x 96-well
171-V26741	Bio-Plex Phospho-Src (Tyr <sup>416</sup> ) Assay, 1 x 96-well
171-V25374**	Bio-Plex Phospho-S6 Ribosomal Protein (Ser <sup>235</sup> /Ser <sup>236</sup> ) Assay, 1 x 96-well
171-V23732	Bio-Plex Phospho-STAT2 (Tyr <sup>689</sup> ) Assay, 1 x 96-well
171-V26952	Bio-Plex Phospho-STAT3 (Ser <sup>727</sup> ) Assay, 1 x 96-well
171-V22552	Bio-Plex Phospho-STAT3 (Tyr <sup>705</sup> ) Assay, 1 x 96-well
171-V25772	Bio-Plex Phospho-STAT6 (Tyr <sup>641</sup> ) Assay, 1 x 96-well
171-V23973	Bio-Plex Phospho-TrkA (Tyr <sup>490</sup> ) Assay, 1 x 96-well
171-V26533	Bio-Plex Phospho-Tyk2 (Tyr <sup>1054</sup> /Tyr <sup>1055</sup> ) Assay, 1 x 96-well

### Bio-Plex Premixed Phosphoprotein Assay Panels

X700000A1B	Bio-Plex Phospho 3-Plex Panel, 1 x 96-well, includes coupled beads, detection antibodies, and lysates for the detection of phosphorylated GSK-3 $\alpha$ / $\beta$ , p70 S6 kinase, and STAT3
X70000CLDZ	Bio-Plex Phospho 3-Plex Panel, 1 x 96-well, includes coupled beads, detection antibodies, and lysates for the detection of phosphorylated ATF-2, NF- $\kappa$ B p65, and p90RSK
X7000001RD	Bio-Plex Phospho 5-Plex Panel, 1 x 96-well, includes coupled beads, detection antibodies, and lysates for the detection of phosphorylated Akt, ERK1/2, I $\kappa$ B- $\alpha$ , JNK, and p38 MAPK

\* For multiplexing capabilities of specific assay combinations, request bulletin 5483, or visit us on the Web at [www.bio-rad.com/bio-plex](http://www.bio-rad.com/bio-plex)

\*\* This assay cannot be multiplexed.

Catalog # Description

### Bio-Plex Total Target Singleplex Assays\*

171-V31075	Bio-Plex Total Akt Assay, 1 x 96-well
171-V31620	Bio-Plex Total ATF-2 Assay, 1 x 96-well
171-V37145	Bio-Plex Total Bcr-Abl Assay, 1 x 96-well
171-V34356	Bio-Plex Total c-Jun Assay, 1 x 96-well
171-V36119	Bio-Plex Total CREB Assay, 1 x 96-well
171-V30438	Bio-Plex Total ERK2 Assay, 1 x 96-well
171-V32238	Bio-Plex Total ERK1/2 Assay, 1 x 96-well
171-V34551	Bio-Plex Total HSP27 Assay, 1 x 96-well
171-V30758	Bio-Plex Total I $\kappa$ B- $\alpha$ Assay, 1 x 96-well
171-V31034	Bio-Plex Total JNK Assay, 1 x 96-well
171-V35340	Bio-Plex Total MEK1 Assay, 1 x 96-well
171-V31336	Bio-Plex Total p38 MAPK Assay, 1 x 96-well
171-V35153	Bio-Plex Total p53 Assay, 1 x 96-well
171-V33535	Bio-Plex Total p90RSK Assay, 1 x 96-well

### Bio-Plex Premixed Total Target Assay Panels

X9000001TK Bio-Plex Total 5-Plex Panel, 1 x 96-well, includes coupled beads, detection antibodies, and lysates for the detection of Akt, ERK1/2, I $\kappa$ B- $\alpha$ , JNK, and p38 MAPK

### Bio-Plex Reagent Kits

171-304004	Bio-Plex Phosphoprotein Detection Reagent Kit, 1 x 96-well
171-304005	Bio-Plex Phosphoprotein Detection Reagent Kit, 10 x 96-well

### Bio-Plex Sample Preparation Kits

171-304011	Bio-Plex Cell Lysis Kit, 1 x 96-well
171-304012	Bio-Plex Cell Lysis Kit, 10 x 96-well

### Bio-Plex Buffer

171-304550	Bio-Plex Phosphoprotein Detection Wash Buffer, 1.5 L, for use with Bio-Plex Pro wash stations
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## Bibliography

Gao Q et al., Simultaneous detection of 12 phosphorylated and 7 total proteins using the Bio-Plex suspension array system, Bio-Rad bulletin 2981 (2004)

Manning G et al., The protein kinase complement of the human genome, Science 298, 1912-1934 (2002)

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