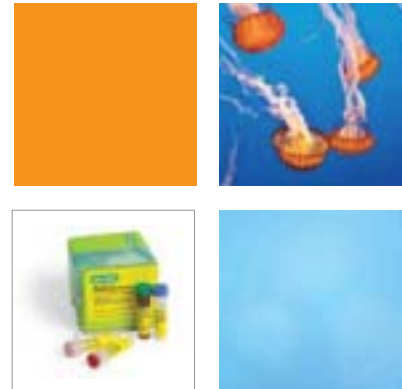




Bio-Plex[®] Phosphoprotein Detection



Explore the Depths



Bio-Plex Phosphoprotein and Total Target Assays

Available Assays

Assays	Phosphoprotein	Total
Akt Signaling		
Akt (Ser ⁴⁷³)	●	●
GSK-3α/β (Ser ²¹ /Ser ⁹)	●	
Cell Cycle/Checkpoint Control		
p53 (Ser ¹⁵)	●	● new
p53 (Ser ³⁷)	● new	● new
p53 (Ser ⁴⁶)	● new	● new
Chromatin Regulation/Acetylation		
Histone H3 (Ser ¹⁰)	●	
Immunology/Inflammation		
IκB-α (Ser ³² /Ser ³⁶)	●	●
NF-κB p65 (Ser ⁵³⁶)	●	
STAT2 (Tyr ⁶⁸⁹)	●	
STAT3 (Ser ⁷²⁷)	● new	
STAT3 (Tyr ⁷⁰⁵)	●	
STAT6 (Tyr ⁶⁴¹)	●	
Tyk2 (Tyr ¹⁰⁵⁴ /Tyr ¹⁰⁵⁵)	● new	
Glucose/Energy Metabolism		
IGF-IR (Tyr ¹¹³¹)	● new	
IR-β (Tyr ¹¹⁴⁶)	● new	
IRS-1 (Ser ⁶³⁶ /Ser ⁶³⁹)	●	
MAP Kinase Signaling		
ATF-2 (Thr ⁷¹)	●	●
c-Jun (Ser ⁶³)	●	●
ERK1 (Thr ²⁰² /Tyr ²⁰⁴)	●	
ERK2 (Thr ¹⁸⁵ /Tyr ¹⁸⁷)	●	●
ERK1/2 (Thr ²⁰² /Tyr ²⁰⁴ , Thr ¹⁸⁵ /Tyr ¹⁸⁷)	●	●
HSP27 (Ser ⁷⁸)	●	●
JNK (Thr ¹⁸³ /Tyr ¹⁸⁵)	●	●
MEK1 (Ser ²¹⁷ /Ser ²²¹)	●	●
p38 MAPK (Thr ¹⁸⁰ /Tyr ¹⁸²)	●	●
p90RSK (Thr ³⁵⁹ /Ser ³⁶³)	●	●
Neuroscience		
CREB (Ser ¹³³)	●	●
TrkA (Tyr ⁴⁹⁰)	●	
Translational Control		
p70 S6 kinase (Thr ⁴²¹ /Ser ⁴²⁴)	●	
S6 ribosomal protein (Ser ²³⁵ /Ser ²³⁶)	●	
Tyrosine Kinases		
Bcr-Abl (Tyr ²⁴⁵)	● new	● new
c-Abl (Tyr ²⁴⁵)	● new	
c-Abl (Tyr ⁴¹²)	● new	
EGFR (Tyr)	●	
PDGF receptor-β (Tyr ⁷⁵¹)	●	
Src (Tyr ⁴¹⁶)	● new	

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

Multiplex Assays for Signal Transduction Research



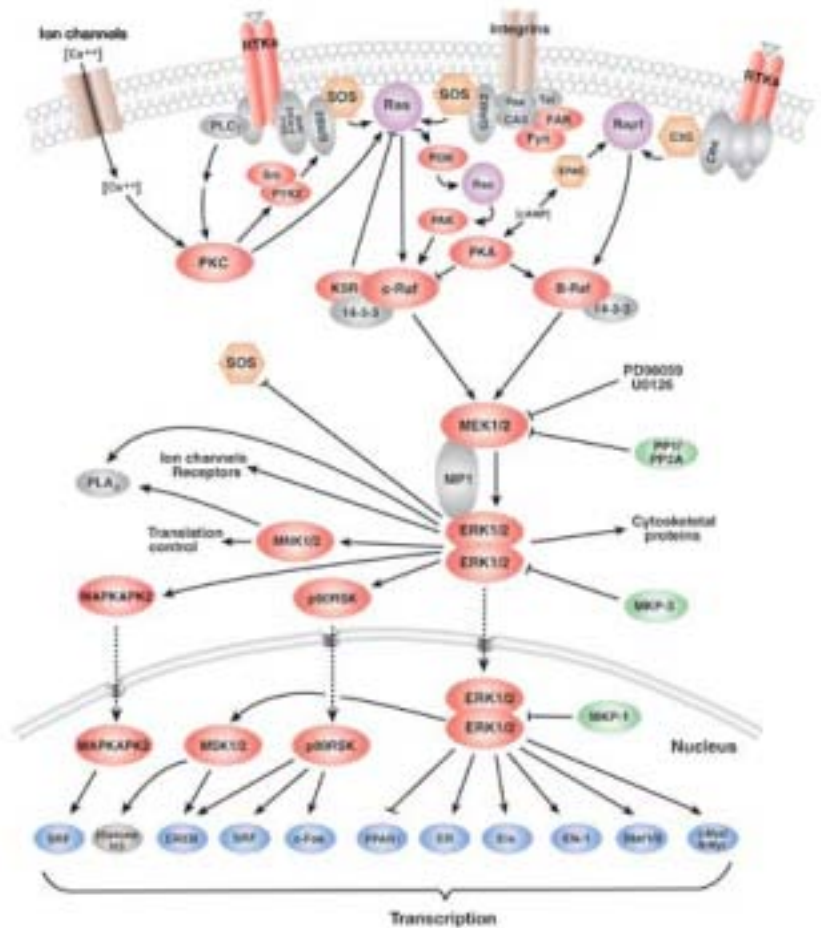
Navigate Signaling Pathways

These assays make it possible to simultaneously examine

multiple phosphorylation events involving upstream or downstream molecules in one or more cellular signaling pathways. Multiplexing thus enables you to maximize the information obtained from a single sample by permitting the analysis of more than one target protein at a time, with minimal sample volume requirements and minimal hands-on time. Whether you're evaluating the phosphorylation states of specific phosphoproteins or the expression levels of total target proteins, it's now quicker and easier to get the data you're looking for.



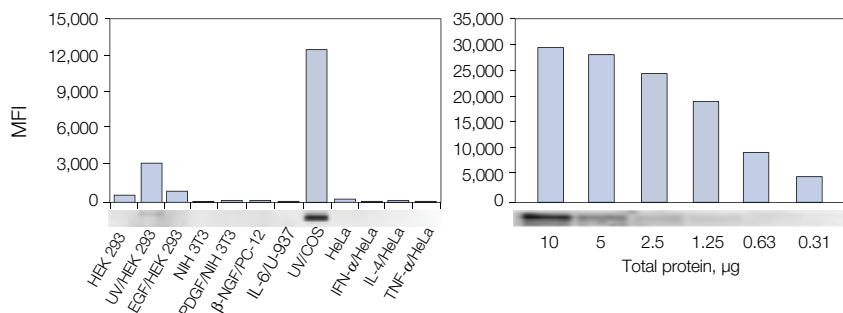
CST antibodies exclusively developed and validated for Bio-Plex phosphoprotein and total target assays.



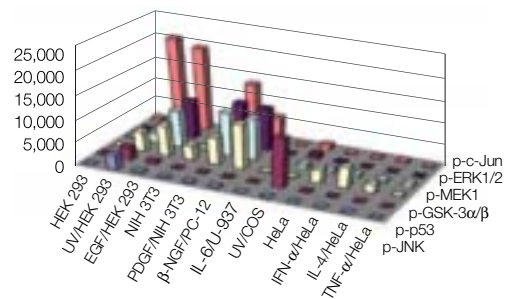
MAPK/ERK in growth and differentiation. Courtesy of CST.

Chart Phosphorylation Events

Bio-Plex phosphoprotein assays and Bio-Plex total target assays are bead-based multiplexable assays that detect the phosphorylation and expression of proteins in lysates derived from cell culture or tissue samples. These 96-well microplate-formatted assays allow profiling of the specific phosphorylation states and expression levels of multiple proteins using only two wells and as little as 25 μ l of lysate per well. 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.



Comparison of Bio-Plex phosphoprotein assays with western blots. Left, detection of phosphorylated p53 (Ser¹⁵) in 12 samples from 6 cell lines with various treatments. Right, detection of phosphorylated p53 in dose-response study of ultraviolet (UV)-treated HT-29 cell lysates. Upper panels, Bio-Plex assays; lower panels, western blots.



Bio-Plex 6-plex phosphoprotein assay analysis.

Activation of phospho-JNK (\blacktriangle), p53 (\blacktriangle), GSK-3 α / β (\blacktriangle), MEK1 (\blacktriangle), ERK1/2 (\blacktriangle), and c-Jun (\blacktriangle) was attenuated in HEK 293, NIH 3T3, PC-12, U-937, COS, and HeLa cells treated with different ligands or stress-related methods.

Limitless Combinations

We are committed to the continual introduction of new assays to increase your multiplexing options. Cell Signaling Technology (CST) is now developing highly specific antibodies exclusively for Bio-Rad's phosphorylation state-specific Bio-Plex assays. This partnership combines Bio-Rad's expertise in bead-based assay development and CST's expertise in antibody development. Check in with us often to stay up-to-date on our new releases!

Leading to Results

Bio-Rad's phosphorylation state-specific Bio-Plex assays feature:

- Highly specific antibodies exclusively developed and validated by CST
- Higher-throughput 96-well microplate format
- Higher-specificity sandwich format that minimizes cross-reaction with other cellular proteins
- Direct measurement of kinases activated within the cell
- Simplified sample preparation with the Bio-Plex cell lysis kit, a kit specifically developed to simplify preparation of cell culture and tissue sample lysates. Lysates prepared with this protocol can be analyzed with any of the Bio-Plex assays or western blotting (request bulletin 3033)

Flexible and Convenient Formats

Bio-Plex phosphoprotein and total target assays are available as singleplex or premixed multiplex assays.

- Singleplex assays are designed to be flexible. You can use them individually to test for a single phosphoprotein at a time, or you can combine them to create a multiplex assay to test for a specific set of phosphoproteins in a single sample
- Premixed multiplex assays are the more convenient format for repeat testing of a specific set of phosphoproteins: The coupled beads are premixed and quality-tested at Bio-Rad, as are the detection antibodies. Custom-premixed assays are available through our online x-Plex™ assay service at www.bio-rad.com/bio-plex/x-plex/

Specifications*

Reactive species	Human, mouse, rat, monkey
Matrices	Cell culture lysate, tissue lysate
Sample volume	25 μ l
Limit of detection	<10 μ g/ml of total protein
Precision	Intra-assay CV <15% Inter-assay CV <25%
Multiplexing capability	Yes
Storage conditions	2–8°C for coupled beads and detection antibodies –20°C for lysates (shipped separately)
Stability**	12 months

* Values vary by assay.

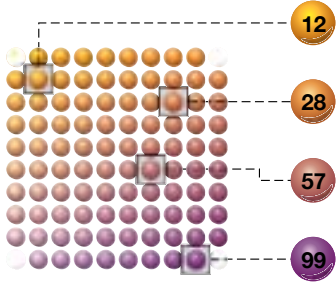
** Check label for expiration date.

What You Need for Phosphoprotein Detection

① **Bio-Plex phosphoprotein assays and Bio-Plex total target assays** — include coupled beads, detection antibodies, and lysates to test for specific proteins of interest in lysate samples.

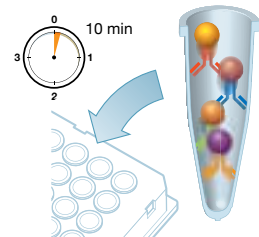
② **Bio-Plex cell lysis kit** — includes cell lysis buffer, cell wash buffer, factor 1, and factor 2; essential for optimal lysis of your cell culture or tissue samples.

③ **Bio-Plex phosphoprotein detection reagent kit** — includes assay buffer, wash buffer, antibody diluent, resuspension buffer, streptavidin-PE, 96-well filter plate, sealing tape, and instructions required to prepare assays and acquire data.



Bio-Plex assays are designed using microscopic beads, each with a different color code (spectral address) to permit discrimination of individual assays. Beads are dyed with different ratios of two fluorophores and are thus classified into 100 unique bead regions (xMAP technology).

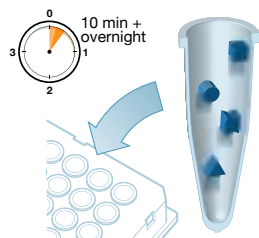
Bio-Plex Immunoassay Workflow For Phosphoprotein and Total Target Assays



1 Coupled Beads

A Bio-Plex assay uses a selection of beads with different spectral addresses, each coupled to antibodies against a different target.

- Add coupled beads to wells of a 96-well plate
- Wash

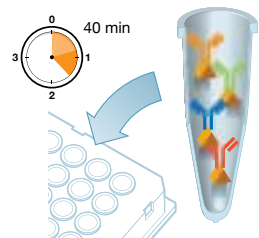


2 Samples

Tissue or cell lysates (prepared with a Bio-Plex cell lysis kit) can be used with Bio-Plex assays.

- Add samples to wells containing coupled beads
- Incubate overnight (or 15–18 hr)
- Wash

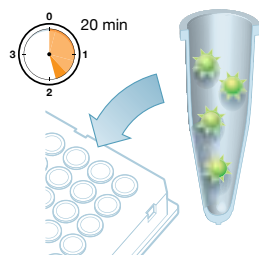
Incubate 15–18 hr



3 Detection Antibodies

Biotin-labeled detection antibodies are specific for secondary epitopes on each target.

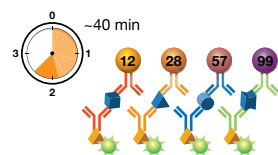
- Add detection antibodies to wells
- Incubate 30 min
- Wash



4 Reporter

Fluorescently labeled streptavidin reporter binds to the biotin-labeled detection antibodies.

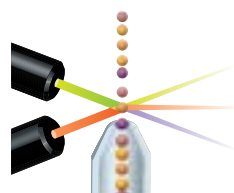
- Add reporter to wells
- Incubate 10 min
- Rinse
- Resuspend in assay buffer



5 Assay

In the Bio-Plex array reader, a red classification laser and a green reporter laser illuminate individual beads to identify each bead's spectral address and associated reporter signal.

- Dyed beads are identified by their internal fluorescent signature (bead region)
- Level of target bound to beads is indicated by intensity of reporter signal
- Multiplex data are reported simultaneously



Ordering Information

Catalog #	Description
Bio-Plex Phosphoprotein Singleplex Assays,* 1 x 96-well	
171-V21075	Bio-Plex Phospho-Akt (Ser ⁴⁷³) Assay
171-V21620	Bio-Plex Phospho-ATF-2 (Thr ⁷¹) Assay
171-V27145	Bio-Plex Phospho-Bcr-Abl (Tyr ²⁴⁵) Assay
171-V27545	Bio-Plex Phospho-c-Abl (Tyr ²⁴⁵) Assay
171-V27745	Bio-Plex Phospho-c-Abl (Tyr ⁴¹²) Assay
171-V24356	Bio-Plex Phospho-c-Jun (Ser ⁶³) Assay
171-V26119	Bio-Plex Phospho-CREB (Ser ¹³³) Assay
171-V23120**	Bio-Plex Phospho-EGFR (Tyr) Assay
171-V21938	Bio-Plex Phospho-ERK1 (Thr ²⁰² /Tyr ²⁰⁴) Assay
171-V20438	Bio-Plex Phospho-ERK2 (Thr ¹⁸⁵ /Tyr ¹⁸⁷) Assay
171-V22238	Bio-Plex Phospho-ERK1/2 (Thr ²⁰² /Tyr ²⁰⁴ , Thr ¹⁸⁵ /Tyr ¹⁸⁷) Assay
171-V23318	Bio-Plex Phospho-GSK-3 α / β (Ser ²¹ /Ser ⁹) Assay
171-V24777	Bio-Plex Phospho-Histone H3 (Ser ¹⁰) Assay
171-V24551	Bio-Plex Phospho-HSP27 (Ser ⁷⁸) Assay
171-V27343	Bio-Plex Phospho-IGF-IR (Tyr ¹¹³¹) Assay
171-V20758	Bio-Plex Phospho-I κ B- α (Ser ³² /Ser ³⁶) Assay
171-V27943	Bio-Plex Phospho-IR- β (Tyr ¹¹⁴⁶) Assay
171-V25576	Bio-Plex Phospho-IRS-1 (Ser ⁶³⁶ /Ser ⁶³⁹) Assay
171-V21034	Bio-Plex Phospho-JNK (Thr ¹⁸³ /Tyr ¹⁸⁵) Assay
171-V25340	Bio-Plex Phospho-MEK1 (Ser ²¹⁷ /Ser ²²¹) Assay
171-V24937	Bio-Plex Phospho-NF- κ B p65 (Ser ⁶³⁶) Assay
171-V21336	Bio-Plex Phospho-p38 MAPK (Thr ¹⁸⁰ /Tyr ¹⁸²) Assay
171-V25153	Bio-Plex Phospho-p53 (Ser ¹⁵) Assay
171-V28353	Bio-Plex Phospho-p53 (Ser ³⁷) Assay
171-V28153	Bio-Plex Phospho-p53 (Ser ⁴⁶) Assay
171-V24155	Bio-Plex Phospho-p70 S6 Kinase (Thr ⁴²¹ /Ser ⁴²⁴) Assay
171-V23535	Bio-Plex Phospho-p90RSK (Thr ³⁵⁹ /Ser ³⁶³) Assay
171-V25957	Bio-Plex Phospho-PDGF Receptor- β (Tyr ⁷⁵¹) Assay
171-V26741	Bio-Plex Phospho-Src (Tyr ⁴¹⁶) Assay
171-V25374**	Bio-Plex Phospho-S6 Ribosomal Protein (Ser ²³⁵ /Ser ²³⁶) Assay
171-V23732	Bio-Plex Phospho-STAT2 (Tyr ⁶⁸⁹) Assay
171-V26952	Bio-Plex Phospho-STAT3 (Ser ²⁷) Assay
171-V22552	Bio-Plex Phospho-STAT3 (Tyr ⁷⁰⁵) Assay
171-V25772	Bio-Plex Phospho-STAT6 (Tyr ⁶⁴¹) Assay
171-V23973	Bio-Plex Phospho-TrkA (Tyr ⁴⁹⁰) Assay
171-V26533	Bio-Plex Phospho-Tyk2 (Tyr ¹⁰⁵⁴ /Tyr ¹⁰⁵⁵) Assay

Bio-Plex Premixed Phosphoprotein Assay Panels

X70000A1B	Bio-Plex Phospho 3-Plex Panel , 1 x 96-well, includes coupled beads, detection antibodies, and lysates for the detection of phosphorylated GSK-3 α / β , 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- κ B p65, and p90RSK
X700001RD	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 κ B- α , 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/

** 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 κ B- α 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 κ B- α , 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

x-Plex Assay Service

These assays are custom-premixed and quality-tested at Bio-Rad. Select your desired combination of Bio-Plex phosphoprotein or total target assays online at www.bio-rad.com/bio-plex/x-plex/ For more information, request bulletin 2905.



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