

Acute Phase Response
Cancer
Cardiovascular Disease
Diabetes
Cytokines, Chemokines,
Growth Factors
Immunology/Inflammation
Immunoglobulin Isotyping
Cell Signaling
Toxicology

Bio-Plex Pro™ Cell Signaling Assays

MAGNETIC SEPARATION ENABLED

Akt, c-Jun, EGFR, Erk1/2, GSK-3 α/β , HER-2, IGF-IR, I κ B- α , JNK, MEK1, NF- κ B p65, p38 MAPK, p70 S6 Kinase, PDGFR- α , PDGFR- β , Smad2, Stat1, Stat3, VEGFR-2

- Magnetic or vacuum assay separation
- Convenient kit format
- Flexible ordering options
- Minimal sample volume
- Antibodies from CST

Cell Signaling Assays with Exceptional Sensitivity

The Bio-Plex Pro cell signaling assays are magnetic bead-based immunoassays designed to meet the sensitivity needs of the most discerning scientists. The multiplex format enables robust, reproducible, and simultaneous measurement of proteins involved in key intracellular signaling pathways. Design your own assay choosing from a broad selection of phosphoprotein and total protein targets to investigate pathways associated with:

- Cancer
- Inflammation
- Diabetes
- Neurological disorders
- Cardiovascular disorders
- Drug mechanism of action
- Toxicology

These assays incorporate several features to enhance both quality and ease of use:

- Assay quick guide to get you started right away
- Assay protocol optimized for exceptional sensitivity and broad dynamic range
- Flexible ordering options — order singleplex sets or visit www.bio-rad.com/assaybuilder to configure a custom premixed kit

Benefits of Magnetic Bead-Based Assays

Magnetic bead-based assays enable automation of wash steps with a Bio-Plex Pro series or similar wash station. This innovation simplifies assay processing and eliminates the need for a vacuum manifold. After adopting the magnetic assay workflow, many users experience improved assay precision, in particular with viscous samples.

Rigorous Assay Validation

All Bio-Plex Pro cell signaling assays undergo a rigorous evaluation that includes the following parameters:

- Specificity and cross-reactivity
- Inter- and intra-assay precision
- Sensitivity (limit of detection, LOD)
- Dynamic range
- Performance characteristics in real samples

Assay Performance Definitions

Precision — the coefficient of variation (%CV) at concentrations within the assay working range

Sensitivity (limit of detection, LOD) — the amount of cell lysate protein for which the fluorescence intensity signal of the specific analyte is two standard deviations above the background signal



BIO-RAD

Available Assays

Assay	Phosphoprotein	Total	Bead Region
Akt (Ser ⁴⁷³)	•	•	75
Akt (Thr ³⁰⁸)	•	•	75
c-Jun (Ser ⁶³)	•	•	56
EGFR (Tyr ¹⁰⁶⁸)	•	•	44
EGFR (Tyr ¹¹⁷³)	•	•	44
Erk1/2 (Thr ²⁰² /Tyr ²⁰⁴ , Thr ¹⁸⁵ /Tyr ¹⁸⁷)	•	•	38
GSK-3 α / β (Ser ²¹ /Ser ⁹)	•	•	18
HER-2 (Tyr ¹²⁴⁸)	•	•	30
IGF-1R (Tyr ¹¹³¹)	•	•	43
I κ B- α (Ser ³² /Ser ³⁶)	•	•	67
JNK (Thr ¹⁸³ /Tyr ¹⁸⁵)	•	•	34
MEK1 (Ser ²¹⁷ /Ser ²²¹)	•	•	27
NF- κ B p65 (Ser ⁵³⁶)	•	•	37
p38 MAPK (Thr ¹⁸⁰ /Tyr ¹⁸²)	•	•	36
p70 S6 Kinase (Thr ⁴²¹ /Ser ⁴²⁴)	•	•	55
p70 S6 Kinase (Thr ³⁸⁹)	•	•	55
PDGFR- α (Tyr ⁷⁵⁴)	•	•	28
PDGFR- β (Tyr ⁷⁵¹)	•	•	57
Smad2 (Ser ⁴⁶⁵ /Ser ⁴⁶⁷)	•	•	14
Stat1 (Tyr ⁷⁰¹)	•	•	61
Stat3 (Ser ⁷²⁷)	•	•	52
Stat3 (Tyr ⁷⁰⁵)	•	•	52
VEGFR-2 (Tyr ¹¹⁷⁵)	•	•	29

Performance Characteristics*

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

* Values vary by assay; precision values are medians of all assays.

Assay Performance

Phosphoprotein Assays	P/B Ratio*	Intra-assay CV%	Inter-assay CV%	Dynamic Range, μ g/well**
Akt (Ser ⁴⁷³)	65	2.9	4.5	0.15–10
Akt (Thr ³⁰⁸)	34	3.3	6.1	0.15–10
c-Jun (Ser ⁶³)	295	5.8	8.4	0.15–10
EGFR (Tyr ¹⁰⁶⁸)	98	14.1	17.7	0.15–10
EGFR (Tyr ¹¹⁷³)	30	3.9	6.3	0.15–10
Erk1/2 (Thr ²⁰² /Tyr ²⁰⁴ , Thr ¹⁸⁵ /Tyr ¹⁸⁷)	48	4.3	4.9	0.15–10
GSK-3 α / β (Ser ²¹ /Ser ⁹)	19	2.9	5.4	0.15–10
HER-2 (Tyr ¹²⁴⁸)	49	10.1	9.1	0.15–10
IGF-1R (Tyr ¹¹³¹)	68	4.8	6.5	0.15–10
I κ B- α (Ser ³² /Ser ³⁶)	103	6.8	8.9	0.15–10
JNK (Thr ¹⁸³ /Tyr ¹⁸⁵)	73	4.7	4.1	0.15–10
MEK1 (Ser ²¹⁷ /Ser ²²¹)	44	1.9	5.5	0.15–10
NF- κ B p65 (Ser ⁵³⁶)	20	10.7	13.5	0.30–10
p38 MAPK (Thr ¹⁸⁰ /Tyr ¹⁸²)	62	3.1	6.4	0.15–10
p70 S6 Kinase (Thr ⁴²¹ /Ser ⁴²⁴)	11	2.9	20.1	0.15–10
p70 S6 Kinase (Thr ³⁸⁹)	196	8.4	10.3	0.15–10
PDGFR- α (Tyr ⁷⁵⁴)	40	5.0	6.2	0.04–10
PDGFR- β (Tyr ⁷⁵¹)	142	8.1	10.3	0.04–10
Smad2 (Ser ⁴⁶⁵ /Ser ⁴⁶⁷)	14	4.4	8.8	0.30–10
Stat1 (Tyr ⁷⁰¹)	18	3.6	7.8	0.15–10
Stat3 (Tyr ⁷⁰⁵)	18	2.5	6.8	0.15–10
Stat3 (Ser ⁷²⁷)	89	7.0	10.1	0.15–10
VEGFR-2 (Tyr ¹¹⁷⁵)	614	8.3	11.7	0.15–10
Total Target Assays	P/B Ratio*	Intra-assay CV%	Inter-assay CV%	Dynamic Range, μ g/well**
Akt	–	3.8	8.4	0.15–10
c-Jun	–	4.0	4.7	0.15–10
Erk1/2	–	3.4	9.2	0.15–10
GSK-3 β	–	2.9	4.1	0.04–2.5
HER-2	–	5.6	4.2	0.04–10
I κ B- α	–	1.9	3.1	0.15–10
JNK	–	1.9	4.5	0.30–10
MEK1	–	1.8	3.9	0.15–10
p38 MAPK	–	4.3	9.6	0.30–10
p70 S6 Kinase	–	2.0	3.6	0.15–10
Smad2	–	3.9	16.9	0.15–10

* Signal ratio (positive/background) of the stimulated cell lysate divided by the negative control cell lysate.

** The range of total lysate protein in which the analyte can be detected.

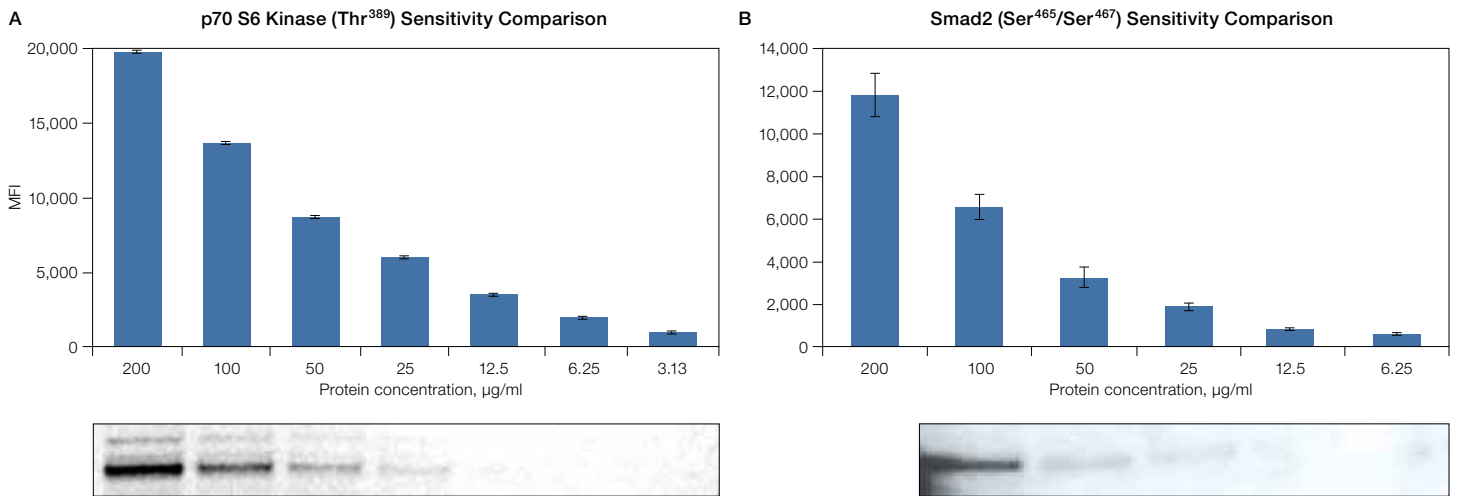


Fig. 1. Bio-Plex Pro cell signaling assays demonstrate greater sensitivity than western blots. **A**, phosphorylated p70 S6 Kinase (Thr³⁸⁹) levels were measured in PC12 cells stimulated with NGFβ. The Bio-Plex assay was able to detect the analyte in as little as 3 µg/ml (150 ng/well) of total lysate protein. **B**, phosphorylated Smad2 (Ser⁴⁶⁵/Ser⁴⁶⁷) levels were measured in HeLa cells stimulated with TNF-α. The Bio-Plex assay was able to detect the analyte in as little as 6 µg/ml (300 ng/well) of total lysate protein.

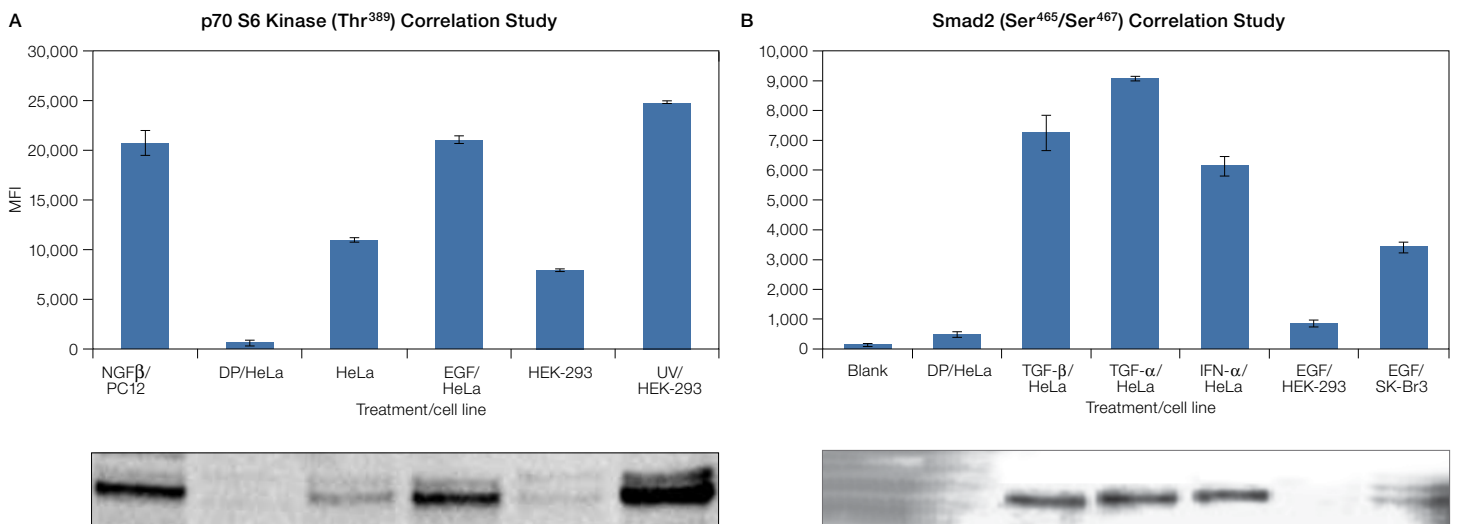


Fig. 2. Performance of Bio-Plex Pro cell signaling assays was compared to that of western blot for various cell lines and treatment conditions. **A**, measurements of p70 S6 kinase (Thr³⁸⁹) in PC12, HeLa, and HEK-293 cell lines. **B**, measurements of Smad2 (Ser⁴⁶⁵/Ser⁴⁶⁷) in HeLa, HEK-293, and SK-Br3 cell lines. Blank indicates detection antibody diluent alone. In both panels A and B, DP/HeLa indicates phosphatase-treated HeLa cells. This condition serves as a negative control.

Flexible Ordering Options

Individual Components

Use the multiplexability chart to select singleplex sets and lysates. Then complete your order with the Bio-Plex Pro cell signaling reagent kit for a mix-it-yourself multiplex solution.

x-Plex™ Custom Assay Service

A great choice for premium custom-mixed assays. Simply select your analytes of interest using the online Bio-Plex assay builder at www.bio-rad.com/assaybuilder. Assays are mixed for you at Bio-Rad and delivered as an all-in-one kit.

Ordering Information

Catalog # Description

Phosphoprotein Singleplex Sets

1 x 96-well, includes coupled magnetic beads and detection antibodies for detecting phosphorylated protein, requires cell signaling reagent kit and optional lysate controls

171-V50001M	Akt (Ser ⁴⁷³)
171-V50002M	Akt (Thr ³⁰⁸)
171-V50003M	c-Jun (Ser ⁶³)
171-V50004M	EGFR (Tyr ¹⁰⁶⁸)
171-V50005M	EGFR (Tyr ¹¹⁷³)
171-V50006M	Erk1/2 (Thr ²⁰² /Tyr ²⁰⁴ , Thr ¹⁸⁵ /Tyr ¹⁸⁷)
171-V50007M	GSK-3α/β (Ser ²¹ /Ser ⁹)
171-V50008M	HER-2 (Tyr ¹²⁴⁸)
171-V50009M	IGF-IR (Tyr ¹¹³¹)
171-V50010M	IκB-α (Ser ³² /Ser ³⁶)
171-V50011M	JNK (Thr ¹⁸³ /Tyr ¹⁸⁵)
171-V50012M	MEK1 (Ser ²¹⁷ /Ser ²²¹)
171-V50013M	NF-κB p65 (Ser ⁵³⁶)
171-V50014M	p38 MAPK (Thr ¹⁸⁰ /Tyr ¹⁸²)
171-V50015M	p70 S6 Kinase (Thr ⁴²¹ /Ser ⁴²⁴)
171-V50016M	p70 S6 Kinase (Thr ³⁸⁹)
171-V50017M	PDGFR-α (Tyr ⁷⁵⁴)
171-V50018M	PDGFR-β (Tyr ⁷⁵¹)
171-V50019M	Smad2 (Ser ⁴⁶⁵ /Ser ⁴⁶⁷)
171-V50020M	Stat1 (Tyr ⁷⁰¹)
171-V50021M	Stat3 (Ser ⁷²⁷)
171-V50022M	Stat3 (Tyr ⁷⁰⁵)
171-V50023M	VEGFR-2 (Tyr ¹¹⁷⁵)

Total Target Singleplex Sets

171-V60001M	Akt
171-V60002M	c-Jun
171-V60003M	Erk1/2
171-V60004M	GSK-3β
171-V60005M	HER-2
171-V60006M	IκB-α
171-V60007M	JNK
171-V60008M	MEK1
171-V60009M	p38 MAPK
171-V60010M	p70 S6 Kinase
171-V60011M	Smad2

Bio-Plex Pro Cell Signaling Reagents

171-304006M	Bio-Plex Pro Cell Signaling Reagent Kit , 1 x 96-well, includes cell lysis buffer, cell lysis factor QG, cell wash buffer, bead resuspension buffer, detection antibody diluent, wash buffer, streptavidin-PE, 96-well flat bottom plate, sealing tape, and instructions
171-304515	Bio-Plex Pro Cell Signaling Wash Buffer , 330 ml, for 1 x 96-well assay, for use with Bio-Plex Pro cell signaling assays only, compatible with both magnetic and vacuum separation methods

Wash Stations

300-34376	Bio-Plex Pro Wash Station , includes magnetic plate carrier, waste bottle, 2 buffer bottles
300-34377	Bio-Plex Pro II Wash Station , includes magnetic plate carrier, vacuum manifold plate carrier, waste bottle, 2 buffer bottles

Accessories

171-020100	Bio-Plex Handheld Magnetic Washer , includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex magnetic assays
171-304502	Filter Plate , pkg of 1, 96-well plate, with clear plastic lid and tray, for Bio-Plex assays using the vacuum wash method, sealing tape not included

Bio-Rad Cell Lysate Controls* – Selection Guide

Phosphoprotein	Lysate Control	Catalog #
Akt (Ser ⁴⁷³)		
Akt (Thr ³⁰⁸)		
Erk1/2 (Thr ²⁰² /Tyr ²⁰⁴ , Thr ¹⁸⁵ /Tyr ¹⁸⁷)	EGF-treated HEK-293	171-YZ0001
GSK-3α/β (Ser ²¹ /Ser ⁹)		
MEK1 (Ser ²¹⁷ /Ser ²²¹)		
c-Jun (Ser ⁶³)		
JNK (Thr ¹⁸³ /Tyr ¹⁸⁵)		
p38 MAPK (Thr ¹⁸⁰ /Tyr ¹⁸²)	UV-treated HEK-293	171-YZ0009
EGFR (Tyr ¹⁰⁶⁸)		
EGFR (Tyr ¹¹⁷³)	EGF-treated HeLa	171-YZ0002
HER-2 (Tyr ¹²⁴⁸)	EGF-treated SK-Br3	171-YZ0003
IGF-IR (Tyr ¹¹³¹)	IGF-1-treated HEK-293	171-YZ0005
IκB-α (Ser ³² /Ser ³⁶)		
NF-κB p65 (Ser ⁵³⁶)	TNF-α-treated HeLa	171-YZ0008
Smad2 (Ser ⁴⁶⁵ /Ser ⁴⁶⁷)		
p70 S6 Kinase (Thr ⁴²¹ /Ser ⁴²⁴)	NGFβ-treated PC-12	171-YZ0006
p70 S6 Kinase (Thr ³⁸⁹)		
PDGFR-α (Tyr ⁷⁵⁴)	PDGF-treated NIH3T3	171-YZ0007
PDGFR-β (Tyr ⁷⁵¹)		
Stat1 (Tyr ⁷⁰¹)		
Stat3 (Ser ⁷²⁷)	IFN-α-treated HeLa	171-YZ0004
Stat3 (Tyr ⁷⁰⁵)		
VEGFR-2 (Tyr ¹¹⁷⁵)	VEGF-treated HUVEC	171-YZ0010
Negative control for all phosphoprotein assays	Phosphatase-treated HeLa	171-YZB001
Total Target	Lysate Control	Catalog #
All total assays except HER-2 and c-Jun	Untreated HeLa	171-YZT002
Total c-Jun	Untreated HEK-293	171-YZT001
Total HER-2	EGF-treated SK-Br3	171-YZ0003
Negative control for all total target assays	Detection antibody diluent**	

* All controls are shipped as one vial of lyophilized cell lysate.

** Included with the Bio-Plex Pro cell signaling reagent kit (171-304006M).

Software

171-001510	Bio-Plex Data Pro™ Software with Bio-Plex Manager™ Software , Bio-Plex Data Pro software (5 seats), for multi-experiment analysis and advanced data visualization, and Bio-Plex Manager software (5 seats), for instrument data evaluation and optimization. CDs and security HASP key included
171-001513	Bio-Plex Data Pro Software , (5 seats), for multi-experiment analysis and advanced data visualization
171-STND01	Bio-Plex Manager Software , includes 1 user desktop license, to analyze Bio-Plex data and generate protocols, does not operate the instrument



CST antibodies developed and validated for Bio-Plex cell signaling, phosphoprotein, and total target assays.

HASP is a trademark of Aladdin Knowledge Systems, Ltd.



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