SIGNAL TRANSDUCTION ASSAYS

Bio-Plex® Phosphoprotein Assays

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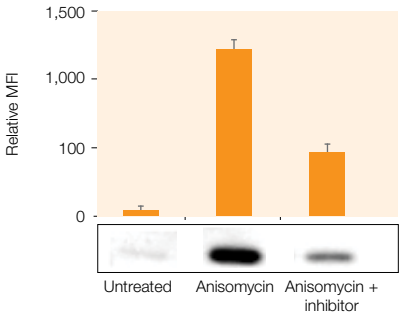
Bio-Plex Phosphoprotein Assays Feature Cell Signaling Technology Antibodies

Assays for Signal Transduction Research

Kinase-mediated protein phosphorylation is a primary signal transduction mechanism utilized in nearly every aspect of cell physiology, including proliferation, differentiation, cell morphology changes, and programmed cell death. Abnormalities in kinase function and cellular signaling underlie many diseases, most notably many cancers, cardiovascular disorders, and metabolic and inflammatory diseases. Thus, understanding kinase biology and the regulation of protein phosphorylation is an important research focus. Because over 500 kinases are encoded by the human genome, it will require highly specific multiplex assays to elucidate the distinct and overlapping functions of kinases in normal and diseased cells. Bio-Rad and Cell Signaling Technology (CST) have partnered to combine their expertise in bead-based assay development and antibody development for comprehensive multiplex analysis of the human kinome. The use of the resulting Bio-Plex phosphoprotein assays to assess the activation of signal transduction pathways is an important application in both basic research and drug discovery.

Phospho-Specific Antibody Specialists

CST is internationally recognized as the premier provider of phosphorylation-specific antibodies, with over 250 phosphorylation-specific antibodies that are produced, purified, quality controlled, and validated by CST scientists. These antibodies are powerful tools for assessing the activation state of proteins and pathways that are critical for basic scientific research, target validation, drug discovery, and clinical pathology. CST is developing and validating antibodies exclusively for Bio-Rad's phosphorylation state-specific Bio-Plex assays. The development and rigorous validation of CST phospho-specific antibodies for the Bio-Plex system will enable investigators to deepen their understanding of the functions of signaling proteins and pathways, and develop targeted therapies to overcome defects in those pathways.



Attenuation of p38 MAPK activation in 3T3 cells treated with a p38 MAPK inhibitor. Upper panel, Bio-Plex phospho-p38 MAPK assay analysis. Lower panel, western blot analysis.

POWERFUL Cell Signaling TECHNOLOGY® CONTENT

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

BIO-RAD

Bio-Plex Phosphoprotein Assay Features

A Direct Approach to Kinase Detection

Cell-based assays enable characterization of protein function and determination of the mode of action of targeted inhibitors. For example, p38 mitogen-activated protein kinase (MAPK) has been implicated in pathological inflammatory responses, and is an important target for therapeutic intervention in a wide array of disorders, including Crohn's disease and rheumatoid arthritis. Traditional cell-based assays examine the production and secretion of various cytokines as surrogate markers, which although functional is many steps downstream of p38 MAPK activation. A direct assay of p38 MAPK would likely provide a more accurate and robust determination of its activity and inhibition. The Bio-Plex phospho-p38 MAPK assay, which uses CST antibodies, is an ideal tool for directly measuring activated p38 MAPK independent of enzyme substrates or other molecules in the activation pathway.

Simultaneous Measurement of Parallel Pathways

Western blotting and flow cytometric analysis are valuable technologies for examining the activation and inhibition of enzymes in lysates and whole cells, respectively, but neither is suitable for higher-throughput analysis. The Bio-Plex phospho-p38 MAPK assay can be readily multiplexed to simultaneously examine numerous phosphorylation-specific endpoints, including parallel MAPK pathways, isoform-specific inhibition, and substrates downstream from p38 MAPK.

Higher Specificity Than Western Blotting

The figure (previous page) demonstrates the attenuation of the p38 MAPK pathway in anisomycin-treated 3T3 cells following p38 MAPK inhibitor treatment as detected by a Bio-Plex assay. These results corroborate those generated by western blotting, indicating effective targeting of p38 MAPK by the inhibitor. Higher specificity is expected with Bio-Plex assays, which combine CST's highly specific antibodies with a two-antibody sandwich approach.

Bio-Rad's expertise in assay development combined with CST's expertise in antibody development will enable the multiplex assessment of many current therapeutic targets in model cell systems and in vivo using the Bio-Plex suspension array system. For more information, request bulletin 2903.

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.

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