



inactive...
or
active?

CASE™ Cellular Activation of Signaling ELISAs

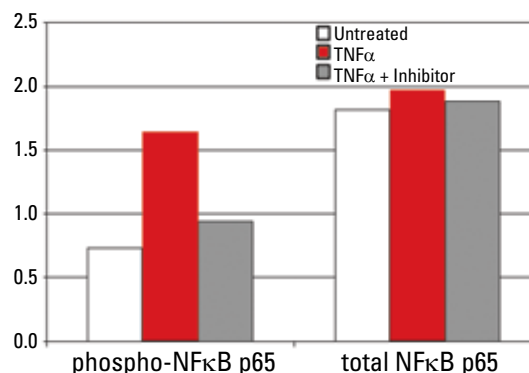
Monitor protein phosphorylation for key signaling pathways.

Quantitative Data without Western Blots

- ❖ **Quantitative:**
Cell-based ELISA determines level of total and phospho-protein at the same time.
- ❖ **Simple:**
Easy, quantitative protocol requires minimal hands-on time with no need for radioactivity.
- ❖ **No More Western Blots:**
Directly measure protein phosphorylation on cells fixed in a 96-well culture plate.

Pathway-Centric Tools and Technology™

NFκB Phosphorylation



Monitoring the activation of NFκB by TNFα. Human A431 cells were either left untreated or were treated with 50 ng/ml TNFα for 5 min. One set of treated cells was pre-treated with the inhibitor Bay 11-7085 at 20 μM for 1 hour. Cells were immediately fixed and analyzed with the CASE Kit for NFκB p65 S536. The relative amount of NFκB p65 phosphorylated at S536 (left) and the relative amount of total NFκB p65 protein (right) are displayed for untreated cells (white bars), for cells with TNFα treatment alone (red bars), and for cells with TNFα treatment after prior treatment with IκBα Inhibitor (gray bars).

CASE™ Cellular Activation of Signaling ELISAs

Inactive... or Active? Directly Monitor Phosphorylation of key signaling proteins on cells cultured in a 96-well plate

Introduction

The cell-based ELISA is a very sensitive and simple method to analyze protein phosphorylation. CASE™ Kits are designed to quantify the phosphorylated form of a protein relative to the total amount of the same protein in cultured cells. By determining protein phosphorylation in your experimental model system, you can verify the activation of pathways in your cell line and determine the effect of various treatments, inhibitors, or activators on the pathway.

CASE Kits overcome the difficulties of traditional Western Blots and radioactive *in vitro* kinase assays, requiring less treatment material with no protein extraction and very little hands-on time. Now you can assay phosphorylation of important proteins in a convenient 96 well plate format that is easily scaled up.

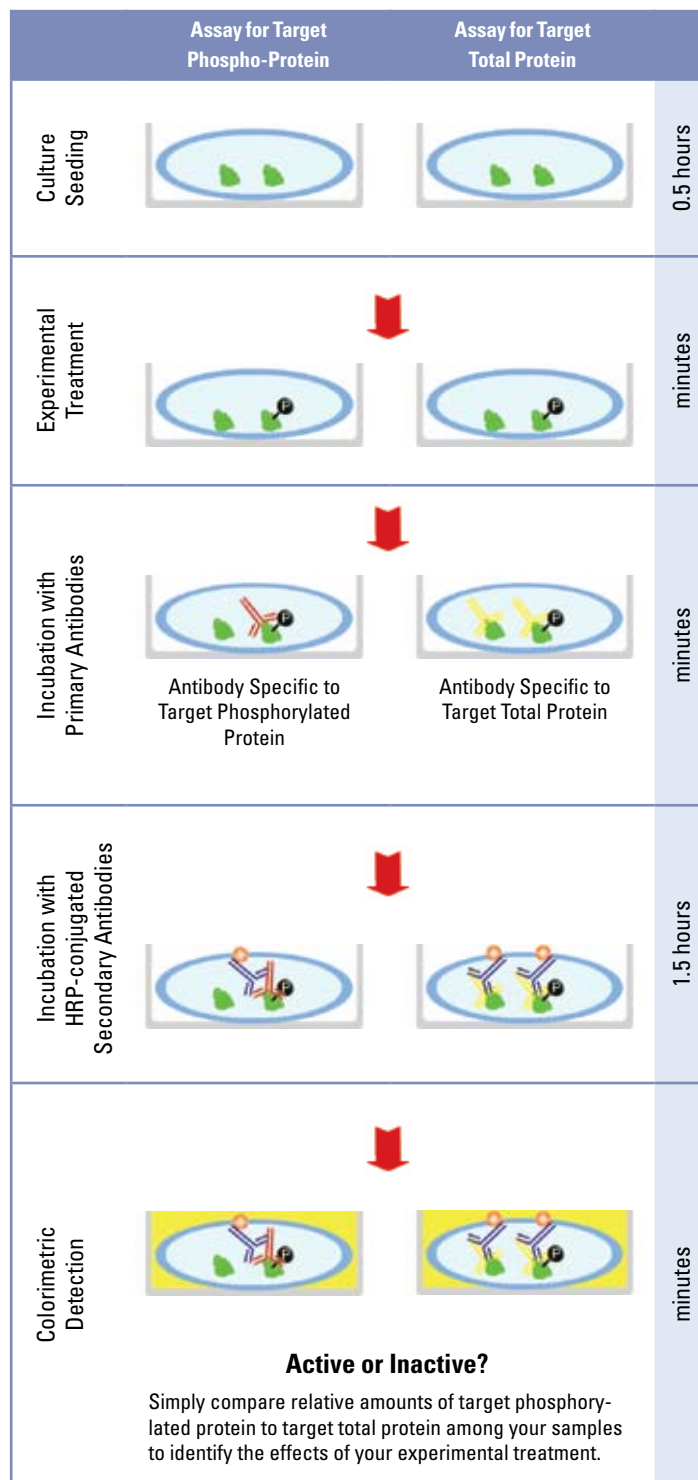


The CASE™ Cellular Activation of Signaling ELISA Kit. Simply grow and treat your cell culture in a 96 well culture plate. In a simple, one day experiment you can determine the amounts of total and phosphorylated protein in your cells directly in the culture plate using the reagents in the CASE™ Kit (plate sold separately).

How It Works



All experimental steps take place in a single 96 well cell culture plate.



CASE™ Kits versus Western Blotting

Comparing CASE™ Kit and Western Blot analysis for detection of AKT phosphorylation induced by IGF-I.

AKT plays a critical role in several signal transduction pathways involved in cell proliferation, apoptosis, angiogenesis, and diabetes. AKT is activated by insulin as well as several growth and survival factors. The CASE™ kit for AKT S473 was used to measure IGF-I induced AKT phosphorylation directly on MCF-7 cells cultured on a 96-well plate without cell lysis. The comparison below shows the quantitative results determined by the CASE™ Kit alongside a Western blot analysis that was carried out in parallel using cell lysate prepared from 6-well cultured cells.

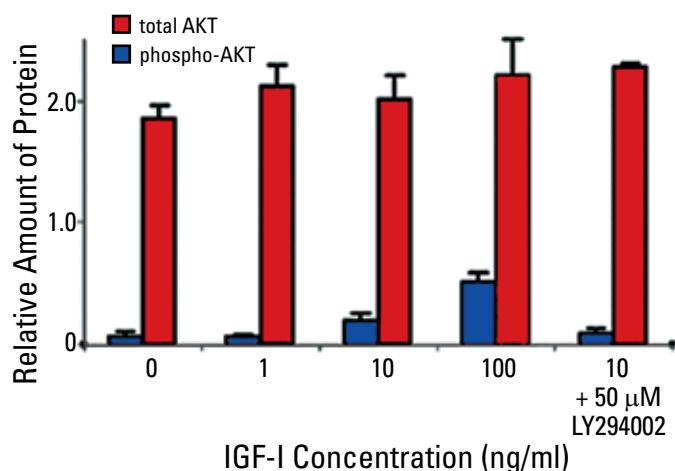


Figure 1A. CASE™ kit analysis of AKT phosphorylation. MCF-7 cells were grown in a 96-well plate and starved before treatment. Cells were treated with AKT activator IGF-I at concentrations of 0, 1, 10, or 100 ng/ml for 30 min, respectively or were pre-treated with inhibitor of LY294002 at a concentration of 50 μM for 2 hours followed by 10 ng of IGF-I for 30 minutes. Phospho-AKT and total AKT levels were measured using the CASE™ Kit for AKT S473 (Cat. No. FE-001).

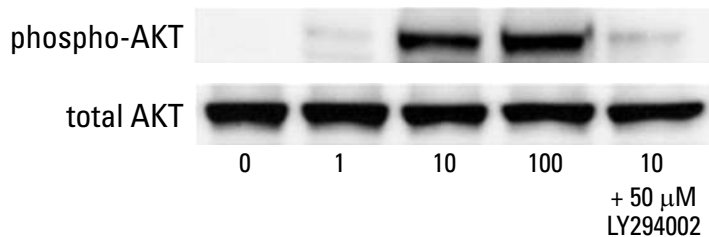


Figure 1B. Western Blot analysis of AKT phosphorylation. MCF-7 cells were grown in a 6-well tissue culture plate and treated with IGF-I and LY294002 as described in Figure 1A. Western blots were used to analyze phospho-AKT and total AKT.

Conclusion

CASE™ kits are an ideal technology for analyzing relative protein phosphorylation directly on 96-well cultured cells. The protein phosphorylation determined by CASE™ is comparable to Western Blot analysis. The cell-based ELISA offers two major benefits over Western Blot analysis: 1. CASE™ kits can significantly save on treatment reagents. Since cells are cultured and analyzed in a 96-well plate, a researcher can use 500 times less treatment reagents with CASE™ kits than what is required in typical Western Blot analyses. This makes CASE™ kits especially suitable for screening different treatment conditions and time courses even when dealing with precious drug treatment reagents. 2. CASE™ kits directly quantify both phosphorylated and total protein at the same time using a conventional ELISA format with colorimetric detection. Unlike Western blots, CASE™ Kits eliminate the need for cell lysis, acrymide gel electrophoresis and membrane blotting and detection.

CASE™ Kits vs. Western Blotting

CASE™ Kits save you time, effort, and reagents: Compare the difference between monitoring phosphorylation by traditional Western Blotting versus new Cell-Based Signaling ELISAs.

Operation	Western (8 assays)	CASE (96 assays)
Harvesting Cells, Extracting Protein	30 min	N/A
Running SDS-PAGE	2 h	N/A
Transfer to Membrane	2 h	N/A
Fixation	N/A	1 h
Blocking	1 h	1 h
Primary Antibody	1 to 18 h	1 h
Washing	30 min	15 min
Secondary Antibody	1 h	1 h
Washing	30 min	15 min
Detection	30 to 60 min	15 min
Data Analysis	1 h	15 min
Total Procedure Time	~ 12 to 26 hours	~ 7 h
Treatment Reagents Required	2 mL per well (6 well plate)	< 100 μL per well (96 well plate)

Using CASE™ kits and Pathway-Focused GEArrays® to characterize activation of the NFκB signaling pathway.

The nuclear factor NFκB is widely recognized as a critical mediator of inflammatory and immune responses. A variety of stimuli (tumor necrosis factor, interleukin 1, T-cell activation signals, bacterial endotoxins, viral transforming proteins, certain growth factors and reactive oxygen intermediates) induce NFκB phosphorylation and subsequent degradation of IκB, leading to the rapid nuclear accumulation of NFκB. In the nucleus, NFκB regulates expression of genes encoding cytokines, cytokine receptors, cell adhesion molecules, proteins involved in coagulation and genes involved in cell growth control. The CASE™ Kit for NFκB S536 was used to assess the phosphorylation state of NFκB and consequent gene expression changes following NFκB activation were determined using the Oligo GEArray® NFκB Signaling Pathway Microarray.

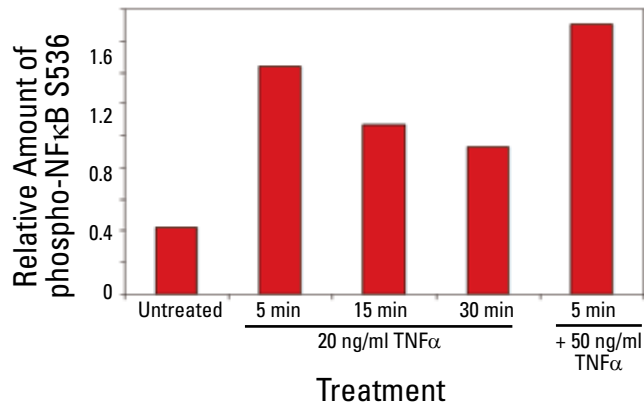


Figure 2A. Monitoring NFκB phosphorylation over a time course. Human A431 cells were grown in 96-well plate and starved before treatment. Cells were treated with 20 ng/ml of NFκB activator TNFα for 5, 15 and 30 min or with 50 ng/ml TNFα for 5 minutes. Phosphorylation levels at Serine-536 of NFκB p65 were measured using the CASE™ kit for NFκB S536 (Cat. No. FE-005).

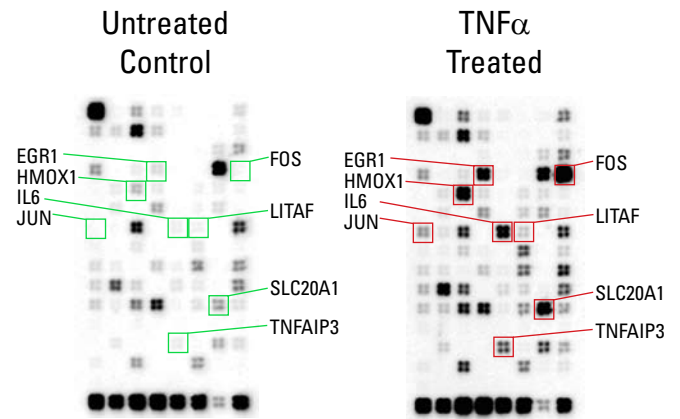


Figure 2B. Determining changes in gene expression by Oligo GEArray®. Human A431 cells were grown in 6-well plate and starved in serum free medium for 18 hours. One set of cells was left untreated and another set of cells were treated with 50 ng/ml TNFα for 5 minutes. Cells were immediately harvested in lysis buffer for RNA isolation. Expression profiles for genes involved in the NFκB signaling pathway were analyzed using the Oligo GEArray NFκB Signaling Pathway Microarray (Cat. No. OHS-025).

Conclusion

CASE™ kits can be used to monitor activation of a signal transduction pathway by measuring phosphorylation of a key mediator of your pathway of interest. In this application example, CASE™ kits quantitatively detected the time course of TNFα induced phosphorylation on Ser-536 of NFκB p65. The activation of NFκB led to induction of NFκB responsive gene expression that can be profiled using the Oligo GEArray NFκB Signaling Pathway Microarray as shown. By combining CASE™ kits and GEArray analysis, researchers can study activation of a signal transduction pathway at both the protein phosphorylation and gene expression levels.

CASE™ Cellular Activation of Signaling ELISAs

CASE™ Kit Target Protein	Activation Site	Species Specificity	CASE™ Kit Catalog Number
ATF2	T69/T71	Mouse Only	FE-020
AKT	S473	Human / Mouse	FE-001
BAD	S112	Human / Mouse	FE-021
BCR	Y177	Mouse Only	FE-019
EGFR	Y845	Human / Mouse	FE-013
ErbB2	Y877	Human / Mouse	FE-012
ERK1/2	T202 / T204	Human / Mouse	FE-002
IκBα	S32	Human Only	FE-008
JNK	T183 / Y185	Human / Mouse	FE-004
JUN	S73	Human / Mouse	FE-009
NFκB p65	S536	Human Only	FE-005
NFκB p65	S468	Human Only	FE-006
NFκB p65	S276	Human Only	FE-007
p38	T180 / Y182	Human / Mouse	FE-003
p53	S9	Human Only	FE-014
p53	S15	Human Only	FE-015
p53	S37	Human Only	FE-016
PIK3R1	YxxM	Human / Mouse	FE-010
SRC	Y418	Human / Mouse	FE-011
STAT3	S727	Human / Mouse	FE-017
STAT3	Y705	Human / Mouse	FE-018

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