

CASE™ Cellular Activation of Signaling ELISA

Directly Measure Protein Phosphorylation without Cell Lysis

CASE™ Kits



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Topics to be Covered

- 1. Introduction to Cell-based Phosphorylation Analysis**
 - Overview of CASE technology
- 2. CASE Experimental Design**
 - Demonstrates how to design an experiment and calculate your results
- 3. Application Data**
 - Three customer success stories

What is CASE?

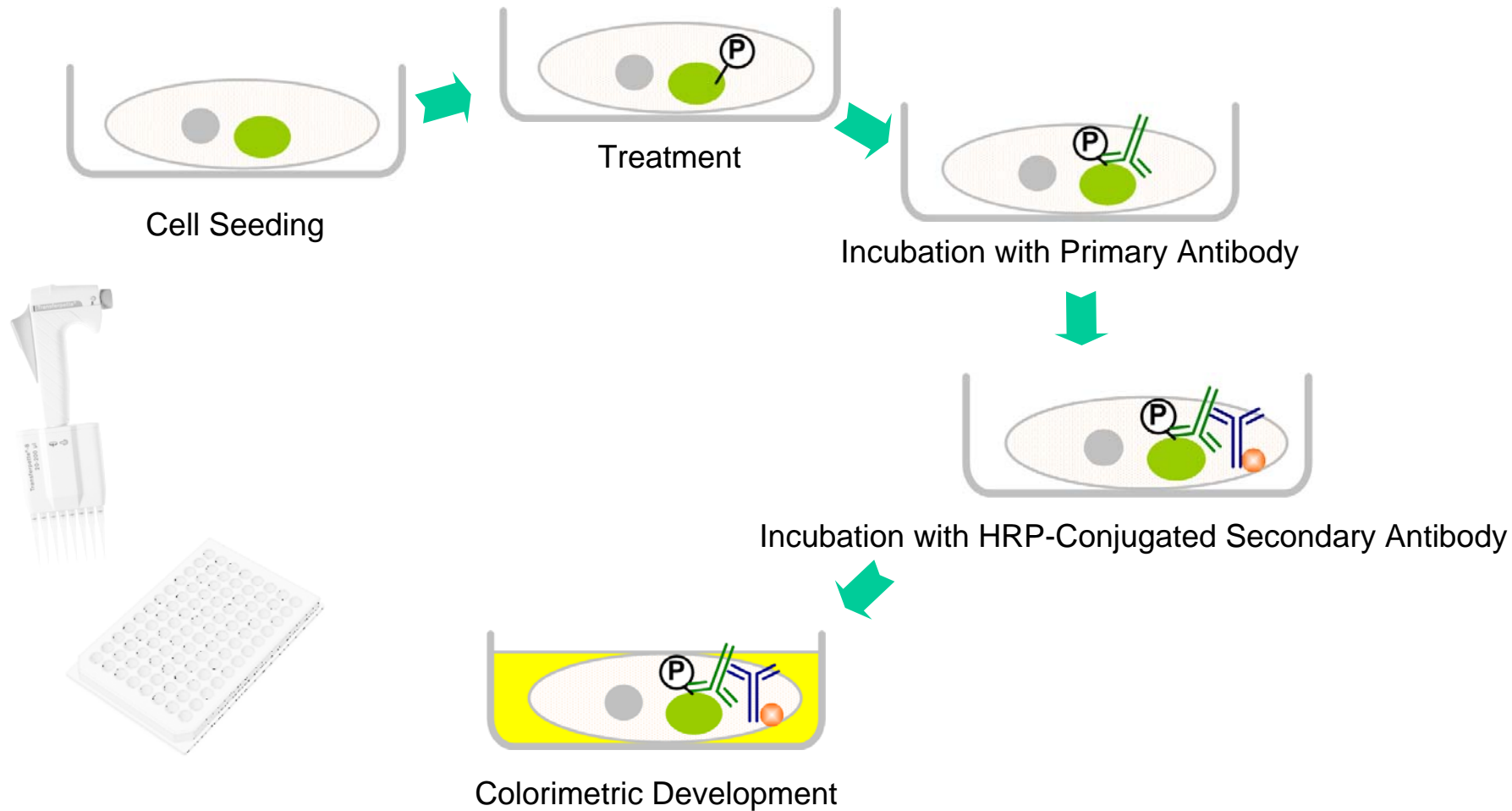
CASE (Cellular Activation of Signaling ELISA):

A cell-based assay designed to quantify the level of phosphorylation of a specific protein relative to the total amount.

A method that quantifies activation of signal transduction pathways.

A kit that includes complete antibody-based detection system.

How Does CASE Work?



Why Use CASE?

CASE is a cell-based assay that eliminates the need for extractions, Western blots and *in vitro* kinase assays.

CASE requires minimum hands-on time.

CASE detects relative amount of total and activated form of a specific protein at the same time.

CASE Kits Currently Available

Protein	Site(s)	Species	Catalog #
AKT	S473	human, mouse	FE-001
ATF2	T69/T71	mouse	FE-020
BAD	S112	human, mouse	FE-021
BCR	Y177	mouse	FE-019
EGFR	Y845	human, mouse	FE-013
ErbB2	Y877	human, mouse	FE-012
ERK	T202/Y204	human, mouse	FE-002
IκBa	S32/36	human	FE-008
JNK	T183/Y185	human, mouse	FE-004
JUN	S73	human, mouse	FE-009

Protein	Site(s)	Species	Catalog #
NFκB p65	S536	human	FE-005
NFκB p65	S468	human	FE-006
NFκB p65	S276	human	FE-007
p38	T180/Y182	human, mouse	FE-003
p53	S9	human	FE-014
p53	S15	human	FE-015
p53	S37	human	FE-016
PIK3R1	YxxM	human, mouse	FE-010
SRC	Y418	human, mouse	FE-011
STAT3	Y705	human, mouse	FE-018
STAT3	S727	human, mouse	FE-017

We are always developing new kits, so please check our [product listing](#) page.

CASE Experimental Design

Sample Experiment: Measure AKT phosphorylation in response to treatment with IGF-1.

Cells are treated with increasing concentrations of IGF-1 with or without the kinase inhibitor LY294002 for 0, 10, 30, or 60 minutes.

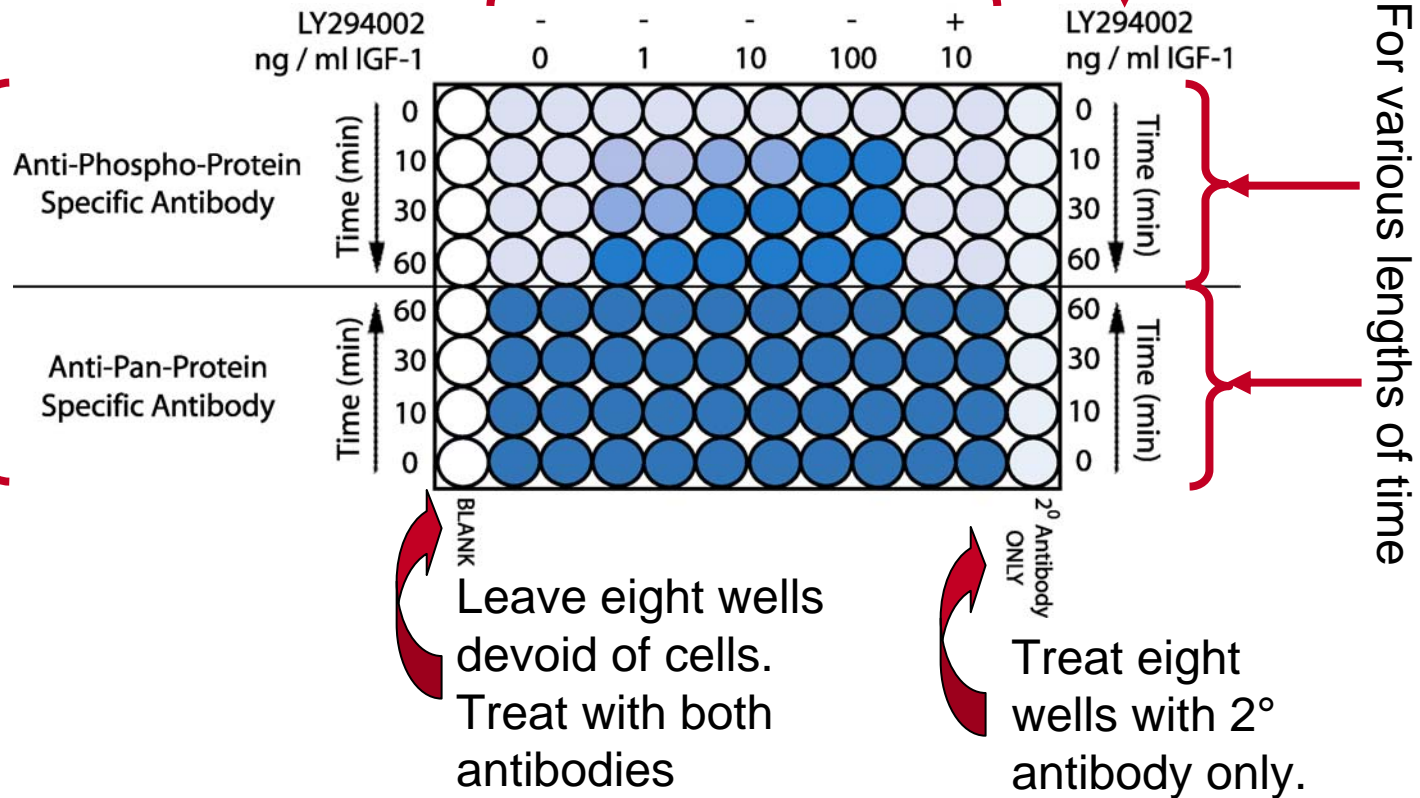
On the next slide is an example plate that would correspond to the above experimental objectives.

Example of Experimental Design

Duplicate wells of cells:

Treat one set with anti-phospho-protein 1° antibody
Treated other set with anti-pan-protein 1° antibody

Treat with various concentrations of IGF-1
With or without the kinase inhibitor LY294002



How does CASE calculate relative phosphorylation?

1. Calculate $A_{450\text{nm}}/A_{595\text{nm}}$ ratio for each well.

This calculation allows the researcher to control for cell number.

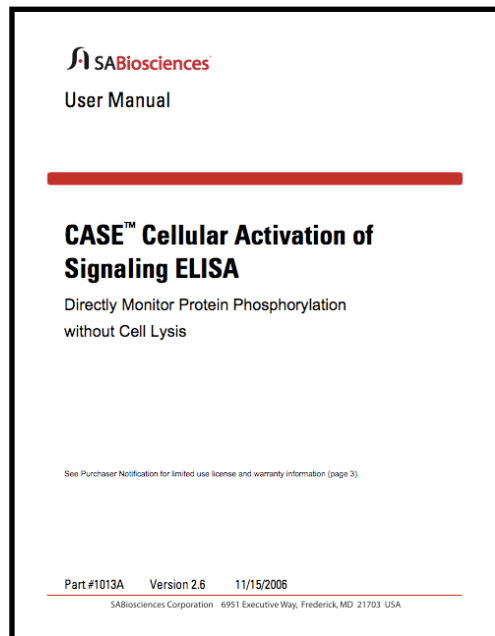
2. Calculate ratio of cell normalized numbers between phospho- and pan-specific antibodies for each sample.

This calculation yields a phosphorylated protein to total protein ratio.

3. Compare total protein normalized numbers to control for each treatment condition.

This calculation determines changes in phosphorylation upon treatment.

CASE Kit Protocol

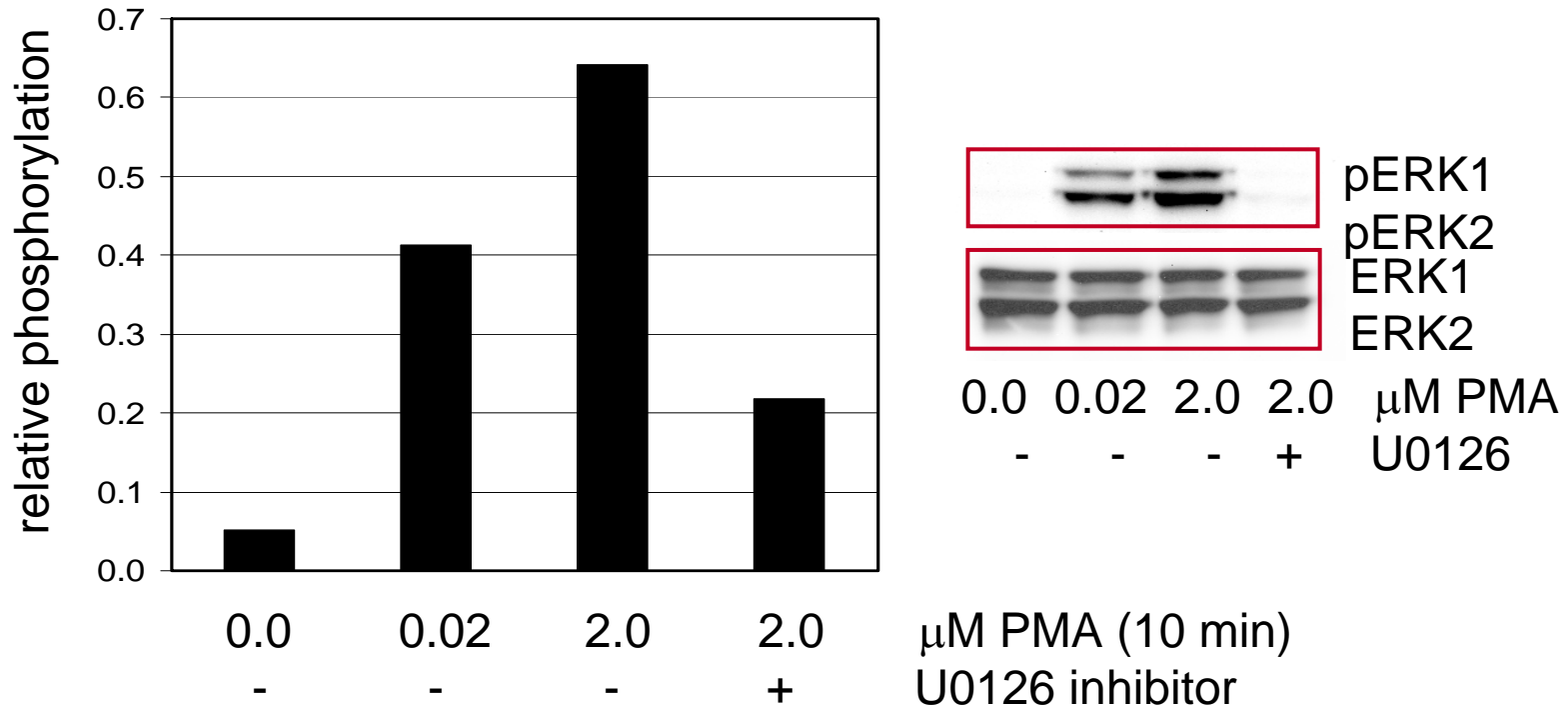


Have more questions about the protocol?

[Click Here for CASE kit User Manual.](#)

Sample Results: Experiment #1

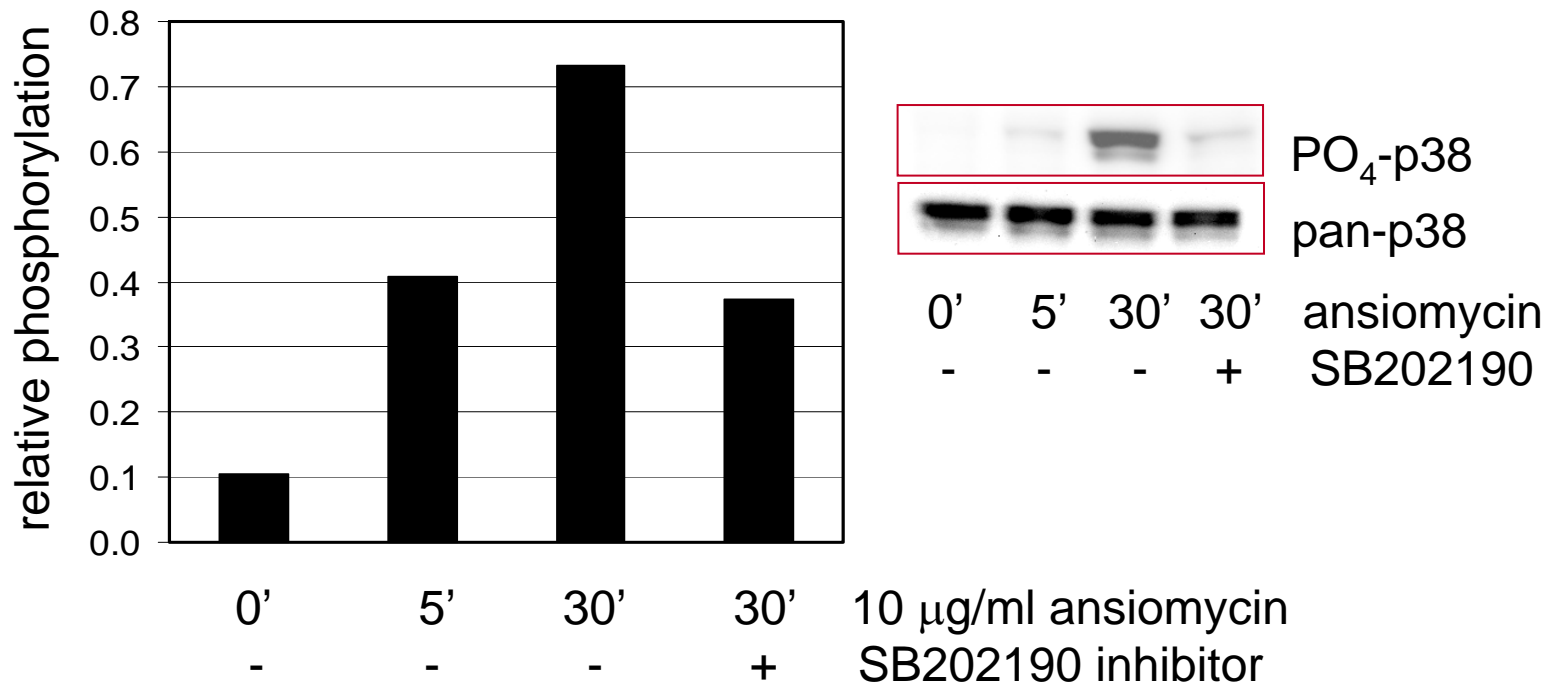
ERK1/2 CASE kit: Activation of ERK by PMA



In this example, A431 cells are treated with the phorbol ester, PMA, either in the presence of absence of an ERK inhibitor, U0126, to activate the ERK signaling pathway. The measurements obtained from the CASE kit demonstrate a dose-dependent increase in phosphorylated-ERK, which is abrogated in the presence of the inhibitor. The right panel is the corresponding Western blot.

Sample Results: Experiment #2

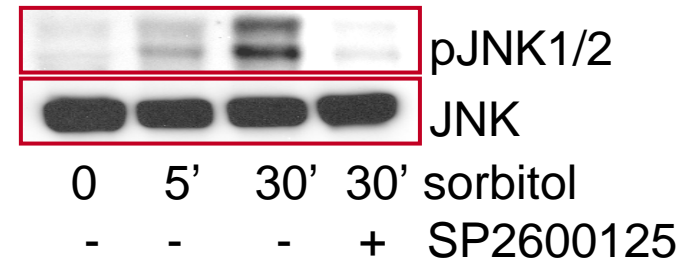
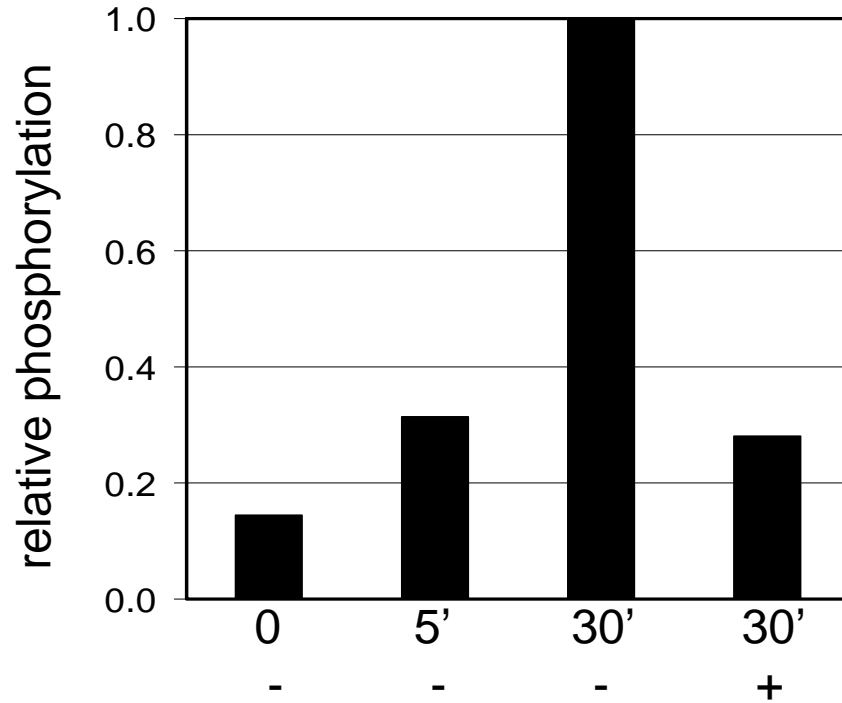
p38 CASE: p38 Activation with Ansiomycin



NIH3T3 cells are treated for increasing time periods with the peptidyl transferase inhibitor, ansiomycin, either in the presence or absence of the p38 inhibitor, SB202190, to activate the p38 signaling pathway. The measurements obtained from the CASE kit demonstrate a time-dependent increase in phosphorylated-p38 in response to ansiomycin treatment, which is abrogated in the presence of the inhibitor. The right panel is the corresponding Western blot.

Sample Results: Experiment #3

JNK CASE: JNK Activation with Sorbitol



0.5 M sorbitol treatment
SP2600125 inhibitor

In this example, A431 cells are treated with sorbitol over time, in the presence or absence of JNK inhibitor, SB202190. Sorbitol is a hyperosmolar stressor to cells which subsequently activate the JNK pathway. The measurements obtained from the CASE kit demonstrate a time-dependent increase in phosphorylated-JNK in response to sorbitol, which is blocked in the presence of the inhibitor. The right panel is the corresponding Western blot.

Summary

Quantitative: CASE does not require additional detection kits or analysis software.

Simple Procedure: CASE requires minimum hands-on time with results in less time than Western blotting.

Direct Measurement: CASE protocol eliminates generating lysates or in vitro kinase assays.