

Product Overview: CASE™ Cellular Activation of Signaling ELISAs

Inactive... or Active? Directly Monitor Protein Phosphorylation Without Cell Lysis

CASE Kits are cell-based ELISAs for measuring kinase phosphorylation. CASE Kits monitor the activation of a signal transduction pathway by assaying the extent to which an important upstream regulatory protein is phosphorylated.

Western blot analysis and radioactive in vitro kinase assays have found widespread use in the monitoring of kinase activation but both methods suffer from distinct disadvantages. These multi-step methods require a large amount of cells and treatment reagents, and are not suitable for high-throughput studies.

CASE Kits overcome these difficulties, requiring 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 for screening experiments.

- **quantitative:** cell-based ELISA determines level of total and activated form of protein at same time
- **simple:** easy, quantitative, non-radioactive protocol with minimal hands-on time
- **no extractions:** directly measure protein phosphorylation state on cells fixed in a 96-well culture plate

Inhibitors for Functional Assays: Testing Specificity of Protein Phosphorylation

The Inhibitors for Functional Assays are designed to help you determine the specificity of the pathway activation observed with a CASE™ Kit. These chemical compounds directly inhibit phosphorylation of the target protein through their upstream kinase. You can test specificity in your CASE assay by simply including control assays in the presence of your experimental stimulus with and without the appropriate inhibitor. If signal is only detected in the absence of the inhibitor, then the upstream regulator specifically activated the pathway.

The inhibitors are provided at convenient stock concentrations. Simply dilute them for use in your CASE assay. Add the dilutions directly to your cell culture medium to pre-treat cells before adding the experimental stimulus under study.

Application Example

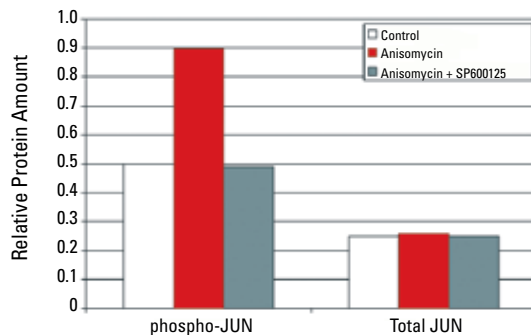
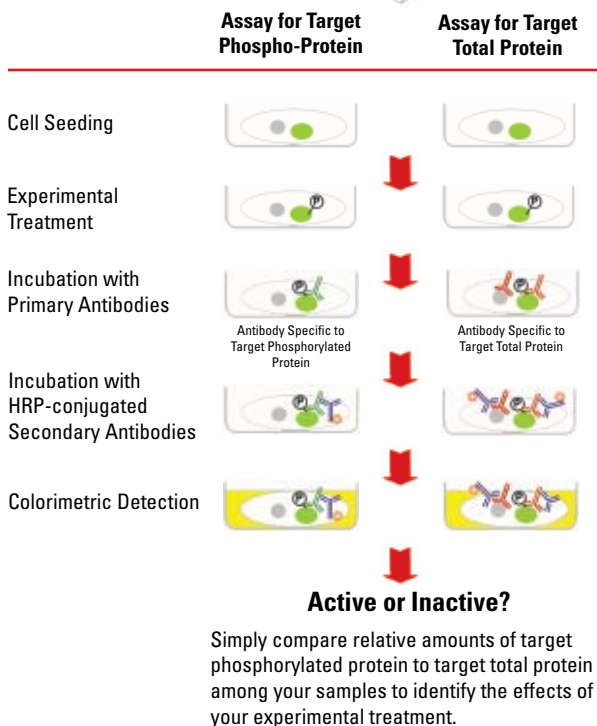


Figure 2: Treatment of A431 cells with Anisomycin Activates JUN. Human A431 cells were starved in serum-free medium for 18 hours. One set of wells was left untreated (white bars), another set was pre-treated with SP600125 (FA-005) at 100 nM for 90 min (gray bars) before treating it with activator and another set (black bars) with 25 µg/ml anisomycin for 30 min. Cells were then immediately fixed and assayed with the CASE Kit for JUN S73 (FE-009). The relative amount of JUN phosphorylated at S73 (left) and the relative amount of total JUN protein (right) are displayed.

How It Works

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



Technical Note: Tips for a Successful CASE™ Experiment

The following are helpful pointers for ensuring successful results with CASE Kits.

Tip #1: Healthy cells are essential for success!

“Healthy” refers to those cells which are growing at log-phase and demonstrate good morphology. Do not use cells over 25 passages. Never use overgrown cells for seeding. They will only waste your time and reagents. Be sure that the cells are at about 80% confluence at the fixing stage.

Tip #2: Don't throw out your cells with your wash solutions!

For loosely adherent cells (such as NIH3T3 and HEK293), take extra care when adding and / or withdrawing medium or treatment solutions. If the opening of your aspiration pipet is too big, cap it with a sterile pipet tip having a smaller opening (e.g. 200 µl tips) to lower the suction force and leave more cells attached to the culture plate. Confine addition and withdrawal of all solutions to one spot in order to minimize the chances of losing cells.

Tip #3: Keep your eye on your cells!

Check the cells under microscope after every step, especially after starvation and fixation. If cell numbers decrease to less than 40% confluence, abort the remainder of the CASE procedure to save your time and antibody. Also, check the nuclei staining status by eye before adding SDS. It should look like sesame seeds spread evenly in each well. If the color of nuclei is too weak, repeat the cell staining step.

Tip #4: Keep your colors safe!

Do you quench fixation with the recommended components and concentrations? Inappropriate quenching inhibits HRP enzyme activity and color development. Do you warm your developing solution to room temperature before colorimetric reaction? Cold solutions slow the enzyme reaction and color development. Do you wash too much? Excessive washing times and additional washing steps can lower signal intensity.

Tip #5: Make your normalization count!

Since we use the OD595 reading to normalize the OD450 readings, it is important to wash out as much excess color residue as possible, so that the OD595 readings accurately reflect the real cell number.

CASE Kit Product Listing

CASE™ Cellular Activation of Signaling ELISAs.

Protein	Activation Site (s)	Species Specificity	CASE Kit Cat. No.	Corresponding Inhibitor
AKT	S473	human, mouse	FE-001	LY294002 (FA-002)
ERK	T202/Y204	human, mouse	FE-002	U0126 (FA-003)
IκBα	S32/S36	human ONLY	FE-008	Bay11-7085 (FA-006)
JNK	T183/Y185	human, mouse	FE-004	SP600125 (FA-005)
JUN	S73	human, mouse	FE-009	SP600125 (FA-005)
NFκB p65	S276	human ONLY	FE-007	Bay11-7085 (FA-006)
NFκB p65	S468	human ONLY	FE-006	Bay11-7085 (FA-006)
NFκB p65	S536	human ONLY	FE-005	Bay11-7085 (FA-006)
p38	T180/Y182	human, mouse	FE-003	SB202190 (FA-004)

Kinase inhibitors for CASE™ Kits and other gene function assays.

Kinase	Product	Inhibitor Cat. No.	Corresponding CASE Kit
IKBKA, IKBKB	Bay11-7085	FA-006	IκBα S32/S36 (FE-008) NFκB p65 (FE-005 to FE-007)
JNK1,2,3	SP600125	FA-005	JNK T183/Y185 (FE-004) JUN S73 (FE-009)
MEK1,2	U0126	FA-003	ERK T202/Y204 (FE-002)
p38 MAPK	SB202190	FA-004	p38 T180/Y182 (FE-003)
PI3K	LY294002	FA-002	AKT S473 (FE-001)