



FACE™

faster phospho-specific
analysis than you ever
thought possible

Fast Activated Cell-base ELISA (FACE™) Kits provide a simple, efficient, cell-based method to monitor protein activation by phosphorylation.

FACE advantages

- Simple, quantitative method that is highly reproducible
- Cell-based assay eliminated the need for cell extracts, gels, blotting and radioactivity
- Fixing cells preserves activation-specific protein modifications
- Minimal hands-on time

Fast Activated Cell-based ELISA (FACE™) Kits provide an efficient, cell-based method to monitor protein activation by phosphorylation. FACE Kits enable modification-specific analysis directly within the cell, without the need for time-consuming cell extractions, gel electrophoresis or membrane blotting. Because the cells are grown, stimulated and assayed in a single 96-well plate, FACE Kits are easily automated. This makes FACE Kits the simplest, most cost-effective phospho-specific assays available.

Many proteins involved in intracellular signaling cascades, like the Mitogen Activated Protein Kinase (MAPK) family of serine/threonine protein kinases, are activated by phosphorylation. These cascades are involved in critical cellular functions, including cell survival, cell differentiation, neural function, cell physiology, inflammation and stress responses. Aberrant control of these signaling cascades is a frequent cause of human cancers and autoimmune diseases. Consequently, there is substantial interest in studying protein activation via phosphorylation. Yet despite its implication in many diseases, protein phosphorylation has traditionally been assayed using low-throughput, labor-intensive approaches.

FACE Kits provide a highly sensitive, 96-well method that overcomes the limitations of in-gel kinase assays and Western blotting. In FACE assays, activated proteins are detected directly within mammalian cells. This eliminates the need to prepare nuclear extracts, run gels and blot membranes. In addition, FACE assays are non-radioactive and generate data that is more quantitative, specific and reproducible than these other methods. FACE Kits are available in both colorimetric and chemiluminescent formats, which enables you to choose the kit that best fits your needs and your in-house equipment.

The FACE method

In the FACE method, cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are fixed, which preserves protein modifications, including phosphorylation. Each well is then incubated with a primary antibody that is specific for the activated protein and a secondary HRP-conjugated antibody. Addition of developing solution provides an easily quantified, colorimetric or chemiluminescent readout. Signals are then normalized for cell number using the provided Crystal Violet solution and plotted (Diagram 1).

Each FACE Kit provides primary antibodies that are specific for both the phosphorylated form of the protein of interest as well as for the total form of the protein. This makes it possible to study phosphorylated protein levels relative to both cell number and the total amount of target protein present within the cells. Because of their novel format, FACE Kits offer one of the most quantitative and reproducible methods available to monitor protein phosphorylation (see Figures 1 & 2, next page).

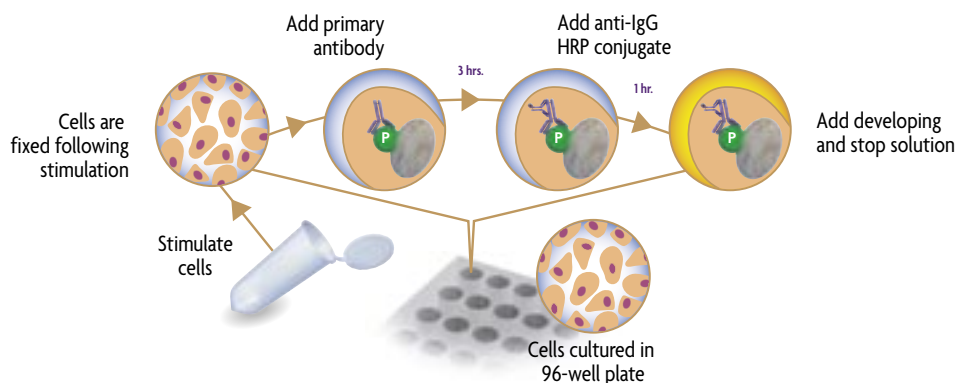


Diagram 1: Flow chart of the FACE procedure.

The FACE advantage

- Simple, quantitative method
- Cell-based assay eliminates extracts, gels, blotting and radioactivity
- Minimal hands-on time
- Primary antibodies to compare phosphorylated & native protein levels
- Compatible with high-throughput automation (and conventional lab equipment)
- FACE Maker enables you to study any phospho-protein of interest

Instant fixation — for accurate results

Preparing cell extracts for Western blotting takes at least an hour to perform. In addition, Western analysis requires determining the protein concentration prior to loading the gel. During this time, additional protein modifications can occur that will alter your results. FACE Kits, however, use a fixation step that “freezes” the cellular state, preventing further protein modifications. This improves your results by enabling you to assay the exact protein state within the cell at your chosen time point.

Quantitative — get the most from your data

Although sensitive, Western blots are more of a qualitative than a quantitative tool. FACE Kits, however, provide results that can be easily quantified relative to the number of cells or to the total amount of target protein present. To illustrate, FACE p38 MAPK assays and Western blots were performed on anisomycin-treated murine macrophage 4/4 cells. FACE Kits clearly yield results that are more quantifiable than Western blotting (Figure 1). Make sure you get the most out of your experiments by using FACE Kits.

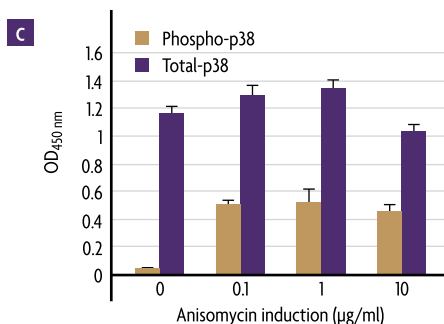
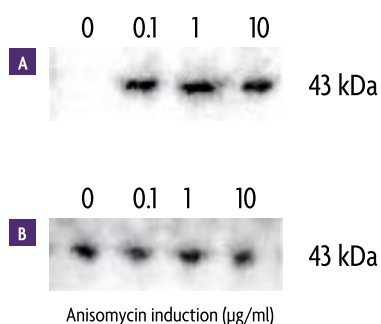


Figure 1: Phospho- and total-p38 MAPK assays.

Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 (B) antibodies. For FACE, 4/4 cells were grown in 96-well plates, stimulated as above, fixed and then assayed in triplicate using the FACE p38 Kit (C). Data were normalized for cell number through use of the kit's Crystal Violet Dye.

Specificity — for confident conclusions

To be certain that you detect only the protein you are interested in, all antibodies included in FACE Kits are stringently tested for cross-reactivity using Western blot analysis. In particular, the phospho-antibodies are assayed to verify that they detect only the activated form of the target protein. The phospho and total antibodies are used in tandem to make sure that the phospho-antibody doesn't interact with other phosphorylated proteins. This ensures that FACE kits are highly specific and detect only the correct protein of interest (Figure 1).

Reproducible — for more meaningful results

FACE Kits are highly reproducible, which is extremely important when measuring small changes in the amounts of a phosphorylated protein. To demonstrate, FACE assays were performed on three different samples of macrophage 4/4 cells that had been treated in an identical manner with PMA to induce ERK phosphorylation. Levels of phosphorylated ERK were highly consistent between each sample (Figure 2). The high level of reproducibility obtained using FACE Kits makes it possible to accurately monitor differences in protein phosphorylation.

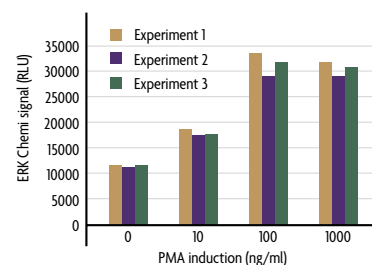


Figure 2: Reproducible assay of phosphorylated ERK.

Murine macrophage 4/4 cells were cultured in 96-well plates and serum starved for 16 hours. Cells were then stimulated with Phorbol 12-myristate 13-acetate (PMA) for 10 minutes and fixed. Levels of phospho ERK were assayed in triplicate using the FACE ERK1/2 Chemi Kit. Data was plotted after normalization for cell number (performed through use of Crystal Violet).

FACE Profiler

Many proteins, such as NFκB, are phosphorylated on multiple residues and can be induced by a variety of stimuli. However, being able to distinguish between different phosphorylation sites can be difficult using conventional methods. Active Motif's FACE Profiler Kits make it possible to rapidly profile the levels of different phosphorylation sites of a protein in one simple experiment.

FACE Maker

FACE Maker is an adaptable version of the FACE Kit, enabling you to study any phosphorylated protein you choose in a simple, fast and sensitive cell-based assay. Using antibodies specific to your desired target protein, modification-state specific analysis is performed directly within the cell, without the need for lysates and time-consuming

Plus, like all FACE Kits, Profiler Kits also include an antibody against the total form of the protein, enabling you to compare phosphorylated to native protein levels. The FACE NFκB p65 Profiler Kit contains antibodies specific for two phosphorylation sites: Serines 468 and 536 (Figure 3). To learn about recent additions to our FACE product line, please visit our website.

immunoblotting. FACE Maker Kits provide all the optimized buffers of our target-specific FACE Kits, but FACE Maker Kits do not include antibodies. So, you are able to study any phosphorylated protein you want with your own antibodies while taking advantage of the effective FACE method and optimized FACE reagents.

Choose from two types of detection

For your convenience, FACE Kits are available in both colorimetric and chemiluminescent formats. The colorimetric kits detect phosphorylated and total protein levels using an HRP-colorimetric signal at a wavelength of 450 nm. Assays are read by spectrophotometry using a standard ELISA-plate reader. Researchers who require maximum sensitivity should try the FACE Chemi Kits. These ultra-sensitive kits use chemiluminescent detection on a luminometer to accurately monitor even the smallest changes in protein phosphorylation (Figure 4). Chemiluminescent detection pro-

vides more flexible measurement parameters than traditional colorimetric kits, enabling detection limits to be adjusted to maintain linearity and ensure that the detection sensitivity is appropriate to the sample type being assayed. FACE Chemi Kits follow the same protocol as the traditional FACE assays. The only difference is the development solution, which requires the Chemi Kits to use a microplate luminometer or CCD camera-coupled imaging system. And, unlike the colorimetric kits, the chemiluminescent signal is stable over several hours so multiple analyses can be completed.

Try FACE Kits today

FACE Kits simplify the measurement of protein phosphorylation. Their cell-based format eliminates the need for cell extracts, gels and blotting, so you'll speed through your assays in record time. Because the cells

are grown, fixed and assayed in the same plate, protein modifications are kept to a minimum, giving you more accurate results. And, as the assay is reproducible, measuring small changes in phosphorylated protein is

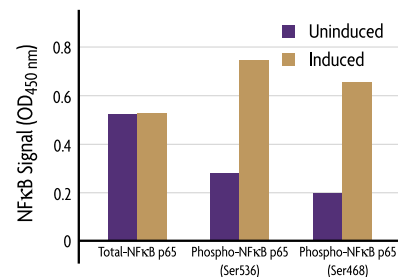


Figure 3: Multi-site Phosphorylation of NFκB p65. FACE NFκB p65 Profiler was used to assay levels of total and phosphorylated NFκB p65 (Ser468 & Ser536) in uninduced and TNF-α + Calyculin A induced HeLa cells. Data was plotted after correcting for the cell number (using the kit's Crystal Violet reagent).

Applications for FACE

The flexibility of FACE Kits make them ideal for a variety of experimental studies, including the monitoring of agonist and antagonist effects on MAPK phosphorylation.

The FACE Kit format has been used successfully with many cell cultures including 3T3-L1, A-431, CHO-K1, COS-7, HeLa, HT-29, Mouse Macrophage, NIH/3T3, Primary rat microglial and Primary human smooth muscle cells.

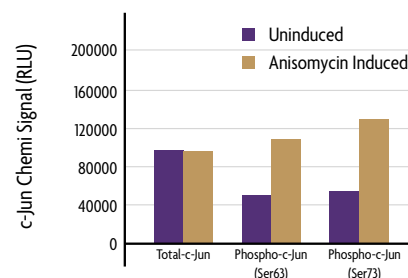


Figure 4: Chemiluminescent detection of c-Jun phosphorylation using FACE Chemi Kits. FACE c-Jun (S63) and c-Jun (S73) Chemi Kits were used to assay total and phosphorylated c-Jun in uninduced and anisomycin-induced NIH/3T3 cells.

actually meaningful. Turn this page over to learn about the many different FACE Kits available, and visit our website to see our latest additions. For simple, efficient analysis of protein phosphorylation, try FACE Kits today.

FACE – Ordering information

Product	Format	Catalog No.
FACE™ Maker	1 x 96 rxns	48000
	5 x 96 rxns	48500
FACE™ Maker Chemi	1 x 96 rxns	48050
	5 x 96 rxns	48550
FACE™ AKT (S473)	1 x 96 rxns	48120
	5 x 96 rxns	48620
FACE™ AKT Chemi	1 x 96 rxns	48220
	5 x 96 rxns	48720
FACE™ ATF-2 (T71)	1 x 96 rxns	48115
	5 x 96 rxns	48615
FACE™ ATF-2 Chemi	1 x 96 rxns	48215
	5 x 96 rxns	48715
FACE™ Bad (S112)	1 x 96 rxns	48165
	5 x 96 rxns	48665
FACE™ Bad Chemi	1 x 96 rxns	48265
	5 x 96 rxns	48765
FACE™ c-Jun (S63)	1 x 96 rxns	48125
	5 x 96 rxns	48625
FACE™ c-Jun Chemi	1 x 96 rxns	48225
	5 x 96 rxns	48725
FACE™ c-Jun (S73)	1 x 96 rxns	48135
	5 x 96 rxns	48635
FACE™ c-Jun Chemi	1 x 96 rxns	48235
	5 x 96 rxns	48735
FACE™ c-Src (Y418)	1 x 96 rxns	48155
	5 x 96 rxns	48655
FACE™ c-Src Chemi	1 x 96 rxns	48255
	5 x 96 rxns	48755
FACE™ EGFR (Y845)	1 x 96 rxns	48340
	5 x 96 rxns	48840
FACE™ EGFR Chemi	1 x 96 rxns	48440
	5 x 96 rxns	48940
FACE™ EGFR (Y992)	1 x 96 rxns	48150
	5 x 96 rxns	48650
FACE™ EGFR Chemi	1 x 96 rxns	48250
	5 x 96 rxns	48750
FACE™ EGFR (Y1173)	1 x 96 rxns	48190
	5 x 96 rxns	48690
FACE™ EGFR Chemi	1 x 96 rxns	48290
	5 x 96 rxns	48790
FACE™ ErbB-2 (Y877)	1 x 96 rxns	48130
	5 x 96 rxns	48630
FACE™ ErbB-2 Chemi	1 x 96 rxns	48230
	5 x 96 rxns	48730
FACE™ ErbB-2 (Y1248)	1 x 96 rxns	48105
	5 x 96 rxns	48605
FACE™ ErbB-2 Chemi	1 x 96 rxns	48205
	5 x 96 rxns	48705
FACE™ ERK1/2 (T202/Y204 & T185/Y187)	1 x 96 rxns	48140
	5 x 96 rxns	48640
FACE™ ERK1/2 Chemi	1 x 96 rxns	48240
	5 x 96 rxns	48740
FACE™ FAK (Y397)	1 x 96 rxns	48145
	5 x 96 rxns	48645
FACE™ FAK Chemi	1 x 96 rxns	48245
	5 x 96 rxns	48745

Product	Format	Catalog No.
FACE™ FKHR (FOXO1) (T24)	1 x 96 rxns	48160
	5 x 96 rxns	48660
FACE™ FKHR (FOXO1) Chemi	1 x 96 rxns	48260
	5 x 96 rxns	48760
FACE™ GSK3β (S9)	1 x 96 rxns	48170
	5 x 96 rxns	48670
FACE™ GSK3β Chemi	1 x 96 rxns	48270
	5 x 96 rxns	48770
FACE™ HSP27 (S82)	1 x 96 rxns	48350
	5 x 96 rxns	48850
FACE™ HSP27 Chemi	1 x 96 rxns	48450
	5 x 96 rxns	48950
FACE™ JAK1 (Y1022/Y1023)	1 x 96 rxns	48185
	5 x 96 rxns	48685
FACE™ JAK1 Chemi	1 x 96 rxns	48285
	5 x 96 rxns	48785
FACE™ JNK (T183/Y185)	1 x 96 rxns	48110
	5 x 96 rxns	48610
FACE™ JNK Chemi	1 x 96 rxns	48210
	5 x 96 rxns	48710
FACE™ MEK1/2 (S217/S221)	1 x 96 rxns	48180
	5 x 96 rxns	48680
FACE™ MEK1/2 Chemi	1 x 96 rxns	48280
	5 x 96 rxns	48780
FACE™ NFκB p65 Profiler (S468, S536)	3 x 96 rxns	48300
	FACE™ NFκB p65 Profiler Chemi	3 x 96 rxns
FACE™ p38 (T180/Y182)	1 x 96 rxns	48100
	5 x 96 rxns	48600
FACE™ p38 Chemi	1 x 96 rxns	48200
	5 x 96 rxns	48700
FACE™ PI3 Kinase p85	1 x 96 rxns	48175
	5 x 96 rxns	48675
FACE™ PI3 Kinase p85 Chemi	1 x 96 rxns	48275
	5 x 96 rxns	48775
FACE™ STAT2 (Y689)	1 x 96 rxns	48310
	5 x 96 rxns	48810
FACE™ STAT2 Chemi	1 x 96 rxns	48410
	5 x 96 rxns	48910
FACE™ STAT4 (Y693)	1 x 96 rxns	48320
	5 x 96 rxns	48820
FACE™ STAT4 Chemi	1 x 96 rxns	48420
	5 x 96 rxns	48920
FACE™ STAT6 (Y641)	1 x 96 rxns	48330
	5 x 96 rxns	48830
FACE™ STAT6 Chemi	1 x 96 rxns	48430
	5 x 96 rxns	48930

CONTENTS & STORAGE

Two, three or ten 96-well plates for culturing cells, primary antibodies (phospho-specific and native protein), secondary antibody, Reaction Buffers and Crystal Violet Cell Quantification Solution. Storage conditions vary from room temperature to -20°C, see manual for details. All reagents guaranteed stable for 6 months when stored properly.