FACE™

faster phospho-specific analysis than you ever thought possible

Fast Activated Cell-based 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 eliminates the need for cell extracts, gels, blotting and radioactivity
- Fixing cells preserves activation-specific protein modifications
- Minimal hands-on time

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

AKT | c-Jun | c-Src | EGFR | ERK | FAK | HSP | JNK | MAPK | NFκB | p38 | pI3K | STAT

FACE™ – accurate, reproducible analysis of protein phosphorylation

Fast Activated Cell-based ELISA (FACE™)* Kits provide a simple, efficient, cell-based method to monitor proteins activated by phosphorylation. FACE Kits enable modification-specific analysis directly within the cell, without the need for cell extractions, gel electrophoresis or membrane blotting. And, 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

FACE Kits are simple to use and require less than 2 hours of hands-on time. 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 activation-specific protein modifications, including phosphorylation. Each well is then incubated with a primary antibody that is specific for the activated protein of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides a colorimetric or chemiluminescent readout that is quantitative and reproducible. The number of cells in each well can be determined easily with the provided Crystal Violet solution, enabling you to normalize for cell number (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. FACE Kits are available for over 20 different targets, or you can develop your own assays with our optimized buffers with FACE Maker. Because of their novel format, FACE Kits offer one of the most quantitative and reproducible methods available to monitor protein phosphorylation (Figures 1 & 2).

* Developed in collaboration with Dr. M. Peppelenbosch and Dr. H. Versteeg.

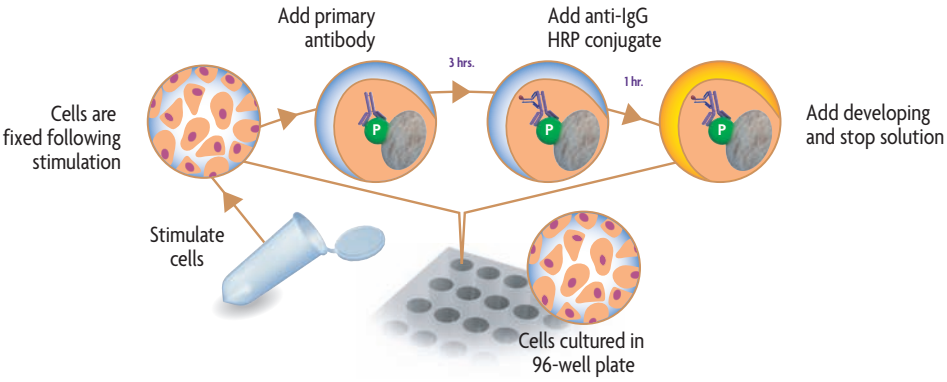


Diagram 1: Flow chart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

FACE™ advantages

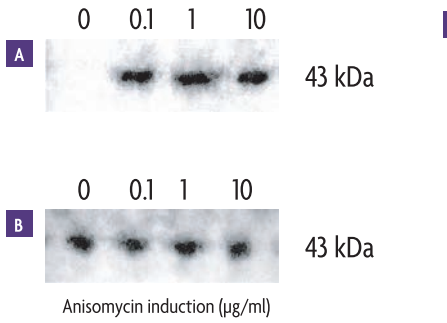
- Simple, quantitative method
- Cell-based assay eliminates extracts, gels, blotting and radioactivity
- Minimal hands-on time
- Plates and antibodies to compare both phosphorylated & total protein levels
- Chemiluminescent sensitivity to detect small changes in phospho-protein levels
- FACE Maker enables you to study any phospho-protein of interest

Rapid fixation – for more precise time points

Preparing cellular extracts for Western blotting is time-consuming, and additional protein modifications can occur during the extraction process that may alter the final results. To eliminate these problems, FACE Kits use a special fixation step that "freezes" the cellular state of the cell and prevents further protein modifications. This enables the detection of the exact protein state within the cell at a chosen time point, which provides you with more accurate results.

Quantitative – get more from your data

Although sensitive, Western blots are more of a qualitative than a quantitative tool. In contrast, FACE Kits 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).



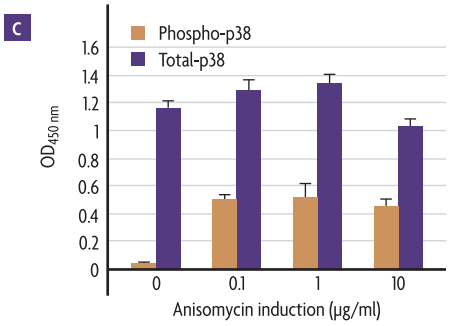


Figure 1: Phospho and total p38 MAPK assays. Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 antibodies (B). For FACE, 4/4 cells were grown in 96-well plates, stimulated as above, fixed and then assayed in triplicate using the FACE p38 Kit (C). Data were corrected for cell number through use of the kit's Crystal Violet Dye. Western blot data provided courtesy of Dr. Henri H. Versteeg and Dr. Maikel P. Peppelenbosch.

Highly specific – for improved accuracy

To be certain that only the protein of interest is detected, all FACE Kit antibodies are stringently tested for cross-reactivity by Western blot analysis. In particular, the phospho-specific 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 does not interact with other phosphorylated proteins. This ensures that FACE Kits are highly specific and detect only the correct protein of interest at the specific phosphorylated site.

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 using FACE Kits makes it possible to accurately monitor subtle differences in protein phosphorylation.

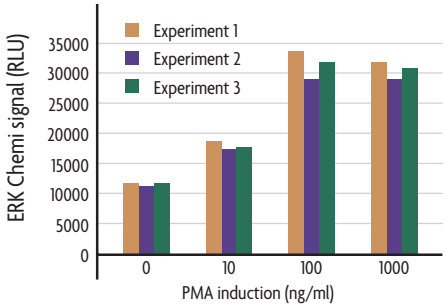


Figure 2: Reproducible assay of phosphorylated ERK. Murine macrophage 4/4 cells were cultured in 96-well plates and serum starved for 16 hours. Cells were then stimulated with Phorbol 12-myristate 13-acetate (PMA) for 10 minutes and fixed. Levels of phospho ERK were assayed in triplicate using the FACE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

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 3). Chemiluminescent detection provides 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.

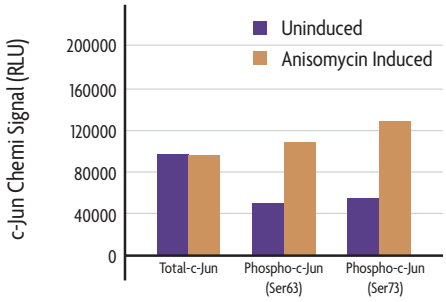


Figure 3: Chemiluminescent detection of phospho c-Jun. FACE c-Jun (S63) and c-Jun (S73) Chemi Kits were used to assay the levels of total and phosphorylated c-Jun contained within uninduced and anisomycin-induced NIH/3T3 cells.

FACE™ NFκB p65 Profiler – study multiple phospho sites

Many proteins, such as NFκB, are phosphorylated on multiple residues and can be induced by a variety of stimuli. Being able to distinguish between different phosphorylation sites on a protein can be difficult to determine using conventional Western blot analysis. The FACE NFκB p65 Profiler Kit makes it possible to rapidly profile phosphorylation levels at Ser468 and Ser536 on NFκB p65 in a single experiment. Plus, like all the FACE Kits, NFκB p65 Profiler includes an antibody against the total form of the NFκB p65, enabling comparisons of phosphorylated to native protein levels (Figure 4).

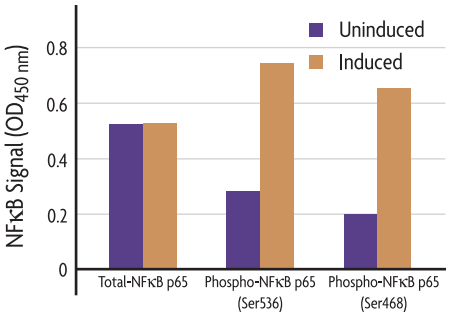


Figure 4: Phosphorylation of NFκB p65 at multiple sites. FACE NFκB p65 Profiler was used to assay levels of total and phosphorylated NFκB p65 in uninduced and TNF-α + Calyculin A induced HeLa cells. Data was plotted after correction for cell number (performed through use of the kit's Crystal Violet reagent).

FACE™ Maker – study any phospho-protein of your choosing

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 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.

Suspension Cell FACE™ – better results with suspension cells

The Suspension Cell FACE module was designed to be used with any colorimetric or chemiluminescent FACE Kit (Figure 5); it provides you with 96-well filter plates that make it easier to perform washing & liquid handling steps when you are working with suspension cells. The filter plates in the module enable you to use a vacuum manifold, which helps eliminate the loss of cells that can occur when performing wash and fixation steps in standard 96-well plates. Because you're able to measure more cells per well, this makes the assay better able to discern small effects.

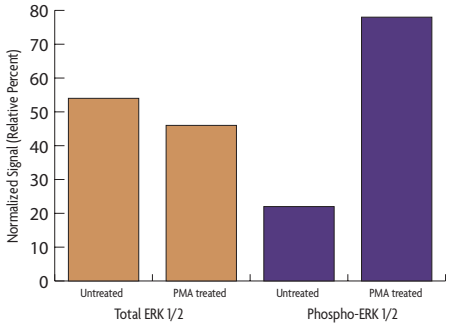


Figure 5: FACE ERK1/2 with the Suspension Cell FACE. The Suspension Cell FACE module was used with FACE ERK1/2 Chemi to assay 50,000 Jurkat cells per well. Jurkat cells were serum-starved and treated with 100 ng/ml PMA (Phorbol 12-myristate 13-acetate) for 15 minutes. A three-fold increase in phospho-ERK1/2 was detected in the assay.

FACE™ – Ordering Information

ACTIVE MOTIF®

FACTOR	PRODUCT	FORMAT	Colorimetric Kit Cat. No.	Chemi Kit Cat. No.
Your choice	FACE™ Maker	1 x 96 rxns	48000	48050
		5 x 96 rxns	48500	48550
Any FACE Kit	Suspension Cell FACE™	2 x 96 rxns	48305	48405
AKT	FACE™ AKT (S473)	1 x 96 rxns	48120	48220
		5 x 96 rxns	48620	48720
ATF	FACE™ ATF-2 (T71)	1 x 96 rxns	48115	48215
		5 x 96 rxns	48615	48715
Bad	FACE™ Bad (S112)	1 x 96 rxns	48165	48265
		5 x 96 rxns	48665	48765
c-Jun	FACE™ c-Jun (S63)	1 x 96 rxns	48125	48225
		5 x 96 rxns	48625	48725
	FACE™ c-Jun (S73)	1 x 96 rxns	48135	48235
		5 x 96 rxns	48635	48735
c-Src	FACE™ c-Src (Y418)	1 x 96 rxns	48155	48255
		5 x 96 rxns	48655	48755
EGFR	FACE™ EGFR (Y845)	1 x 96 rxns	48340	48440
		5 x 96 rxns	48840	48940
	FACE™ EGFR (Y992)	1 x 96 rxns	48150	48250
		5 x 96 rxns	48650	48750
	FACE™ EGFR (Y1173)	1 x 96 rxns	48190	48290
		5 x 96 rxns	48690	48790
ErbB	FACE™ ErbB-2 (Y877)	1 x 96 rxns	48130	48230
		5 x 96 rxns	48630	48730
	FACE™ ErbB-2 (Y1248)	1 x 96 rxns	48105	48205
		5 x 96 rxns	48605	48705
ERK	FACE™ ERK1/2 (T202/Y204 and T185/Y187)	1 x 96 rxns	48140	48240
		5 x 96 rxns	48640	48740
FAK	FACE™ FAK (Y397)	1 x 96 rxns	48145	48245
		5 x 96 rxns	48645	48745
FKHR	FACE™ FKHR (FOXO1) (T24)	1 x 96 rxns	48160	48260
		5 x 96 rxns	48660	48760
HSP	FACE™ HSP27 (S82)	1 x 96 rxns	48350	48450
		5 x 96 rxns	48850	48950
JAK	FACE™ JAK1 (Y1022/Y1023)	1 x 96 rxns	48185	48285
		5 x 96 rxns	48685	48785
JNK	FACE™ JNK (T183/Y185)	1 x 96 rxns	48110	48210
		5 x 96 rxns	48610	48710
MEK	FACE™ MEK1/2 (S217/S221)	1 x 96 rxns	48180	48280
		5 x 96 rxns	48680	48780
NFκB	FACE™ NFκB p65 Profiler (S468 and S536)	3 x 96 rxns	48300	48400
p38	FACE™ p38 (T180/Y182)	1 x 96 rxns	48100	48200
		5 x 96 rxns	48600	48700
PI3K	FACE™ PI3 Kinase p85	1 x 96 rxns	48175	48275
		5 x 96 rxns	48675	48775
STAT	FACE™ STAT2 (Y689)	1 x 96 rxns	48310	48410
		5 x 96 rxns	48810	48910
	FACE™ STAT4 (Y693)	1 x 96 rxns	48320	48420
		5 x 96 rxns	48820	48920
	FACE™ STAT6 (Y641)	1 x 96 rxns	48330	48430
		5 x 96 rxns	48830	48930

CONTENTS & STORAGE

Two, three or ten 96-well plates for culturing cells, primary antibodies (1 phospho-specific and 1 for total protein), HRP-conjugated 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 are guaranteed stable for 6 months when stored properly.