# FACE™

## EGFR ELISA Kits

(version D)

Catalog Nos. 48150 & 48650 (EGFR (Y992))
Catalog Nos. 48250 & 48750 (EGFR (Y992) Chemi)
Catalog Nos. 48190 & 48690 (EGFR (Y1173))
Catalog Nos. 48290 & 48790 (EGFR (Y1173) Chemi)
Catalog Nos. 48340 & 48840 (EGFR (Y845))
Catalog Nos. 48440 & 48940 (EGFR (Y845) Chemi)


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## Overview

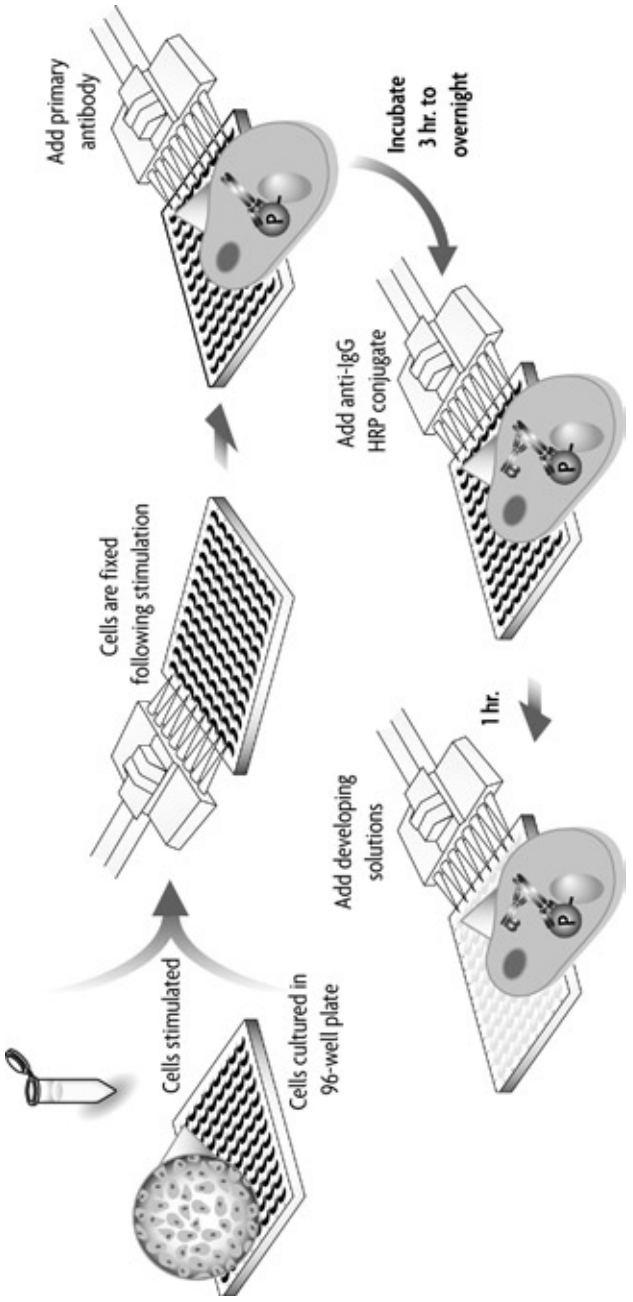
Epidermal Growth Factor (EGF) Receptor (EGFR, HER-1, c-ErbB-1) is a member of the Epidermal Growth Factor Receptor family of Receptor Tyrosine Kinases. These cell surface receptors play an important role in the flow of information from the outside of a cell to the inside. Upon binding of EGF to the extracellular domain, the receptor undergoes dimerization and becomes phosphorylated on several tyrosine residues within the cytoplasmic domain. These result in EGFR activation and increased tyrosine kinase activity toward a variety of intracellular substrates. Autophosphorylation of tyrosine 845, 992 and 1173 are critical to EGFR signaling. Phosphorylation at tyrosine 992 creates a direct binding site for phospholipase C-γ (PLC-γ). PLC-γ binding to EGFR (Y992) results in the activation of protein kinase C and the subsequent activation of downstream signaling cascades.[1] Phosphorylation at 1173 creates a major binding site for the protein tyrosine phosphatase SHP-1, which can dephosphorylate the EGFR and thereby block EGFR-induced activation of the ERK1/2 signaling pathway.[2] Tyrosine 845 phosphorylation is mediated by integrin engagement and Src, and regulates receptor function and tumor progression. Aberrant regulation of EGFR signaling has been associated with many types of cancer including mammary, ovarian, non-small cell lung, glioblastoma, prostate, pancreas and head and neck.[3] Thus, methods to rapidly quantify receptor activation are in high demand.

Fast Activated Cell-based ELISA (FACE™)* Kits provide a simple, efficient, cell-based method to monitor proteins activated by phosphorylation.[4] FACE EGFR Kits are designed specifically to quantify activated (phosphorylated) EGFR and/or total EGFR. In the FACE method, cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are rapidly fixed to preserve activation-specific protein modifications. Each well is then incubated with a primary antibody that recognizes either phosphorylated EGFR or total EGFR. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric or chemiluminescent readout. The relative number of cells in each well is then determined using the provided Crystal Violet solution. The 96-well plate format is suitable for high-throughput screening applications. FACE EGFR Kits are available in both colorimetric and chemiluminescent formats for studying the Y992, Y1173 or Y845 phosphorylation sites of EGFR:

| product | format | catalog no. |
|---|---|---|
| FACE EGFR (Y992) | 1 x 96 rxns | 48150 |
| | 5 x 96 rxns | 48650 |
| FACE EGFR (Y992) 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 (Y1173) Chemi | 1 x 96 rxns | 48290 |
| | 5 x 96 rxns | 48790 |
| FACE EGFR (Y845) | 1 x 96 rxns | 48340 |
| | 5 x 96 rxns | 48840 |
| FACE EGFR (Y845) Chemi | 1 x 96 rxns | 48440 |
| | 5 x 96 rxns | 48940 |

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

## Flow Chart of Process



## Introduction

### EGFR

The Epidermal Growth Factor Receptor (EGFR, HER-1, c-ErbB-1) is a 170 kDa member of the Epidermal Growth Factor (EGF) Receptor family of Receptor Tyrosine Kinases (RTKs) which includes ErbB-2 (Her2/neu), ErbB-3 (HER3) and ErbB-4 (HER4). These cell-surface receptors are glycoproteins comprised of an extracellular ligand-binding domain, a single transmembrane domain and an intracellular domain with ligand-activated tyrosine kinase activity.[5] These proteins modulate critical cellular processes such as mitogenesis, cell death, angiogenesis and cell differentiation.[3, 6, 7]

Proteins of the EGFR family of RTKs are activated by ligand binding. This results in receptor dimerization, autophosphorylation, activation of downstream signaling molecules and ultimately, lysosomal degradation of the receptor.[8, 9] Natural ligands that activate EGFR are EGF and TGF-alpha.

EGFR activity is modulated to a large extent through phosphorylation of several phosphorylation and autophosphorylation sites. Autophosphorylation at Tyr992 creates a direct binding site for the SH2 domain of phospholipase C-γ (PLC-γ). PLC-γ binding to EGFR Y992 results in the activation of protein kinase C (PKC) and subsequent activation of the MAP kinase/ERK signaling cascades.[1] These cascades in turn regulate a variety of proteins, including cytosolic proteins involved in protein translation and nuclear proteins such as the transcription factors Elk-1, c-Jun, STAT1 and 3, Ets-1, c-Myc, ER, CREB and PPARγ.[10-12] Phosphorylation at Tyr1173 permits binding of the N-terminal SH2 domain for the protein tyrosine phosphatase SHP-1. SHP-1 has the capacity to activate the cytoplasmic tyrosine kinase c-Src by dephosphorylation of the inhibitory phosphotyrosine at the C-terminus, an event that may be related to SHP-1-dependent activation of the MAP kinase cascade.[2] Tyrosine 845, a major autophosphorylation site in EGFR, is mediated by integrin engagement and Src, and regulates receptor function and tumor progression.[13] Phosphorylation of Tyr845 in the kinase domain may stabilize the activation loop, maintain the enzyme in an active state and provide a binding surface for substrate proteins.[14,15] c-Src is involved in phosphorylation of Tyr845. [16]

Aberrant control of EGFR is implicated in development, progression and severity of several human cancers. For example, EGFR overexpression correlates with poor prognosis in breast, ovarian and head/neck cancers.[6, 17, 18] Several mutant forms of the coding gene have also been found. ΔEGFR, a deletion mutation lacking exons 2-7, is most common and is associated with glioblastoma.[19]

## Traditional Kinase Assays

To date, two methods are widely used to perform kinase assays:

1. One method used is the in-gel kinase assay, which is an activity staining technique used to study protein kinases.[20] A given protein substrate is immobilized on a gel and phosphorylated by protein kinases, which are separated by SDS-PAGE. The bands of incorporated [$^{32}$P]phosphate are then visualized by autoradiography. While this method is sensitive, it is also cumbersome and is not suitable for high-throughput applications. In-gel kinase assays also require special precautions and equipment for handling radioactivity.
2. Another method used is Western blot analysis. Western blots are performed using antibodies that recognize only the phosphorylated version of the protein of interest. Although less tedious than in-gel kinase assays, Western blotting, like in-gel kinase, requires the preparation of nuclear or whole-cell extract and separation by SDS-PAGE. Furthermore, this process is expensive due to the large quantity of phospho-specific antibody required.

## FACE EGFR

EGFR is heavily studied in both basic and pharmaceutical research because aberrant EGFR control is associated with numerous types of human cancer, and its presence on the cell surface makes it accessible to therapeutic intervention. However, efforts to study EGFR signaling have been hampered by the lack of convenient and high-throughput assays suitable for quantifying EGFR activation (phosphorylation).

To overcome this, Active Motif has introduced FACE EGFR to its line of FACE™ (Fast Activated Cell-based ELISA) Kits. These are highly sensitive 96-well assays designed for detecting activated proteins within mammalian cells. Unlike Western blot, FACE assays do not require cell extracts, electrophoresis or membrane blotting. And, unlike typical kinase assays, FACE assays are non-radioactive and simple to perform. Each FACE EGFR Kit contains two 96-well plates and two primary antibodies. The FACE phospho-EGFR (Tyr992), phospho-EGFR (Tyr1173) and phospho-EGFR (Tyr845) antibodies were raised in rabbits against synthetic phospho-peptides corresponding to residues surrounding phosphorylated Tyr992, Tyr1173 or Tyr845 of human EGF receptor. The phospho-EGFR (Tyr992), phospho-EGFR (Tyr1173) and phospho-EGFR (Tyr845) antibodies recognize EGFR only when phosphorylated at Tyr992, Tyr1173 and Tyr845 respectively. The total-EGFR antibody supplied in the FACE EGFR Kits recognizes EGFR protein regardless of its phosphorylation site.

FACE EGFR Kits can be used to study phosphorylated EGFR relative to cell number. In this application, cells are cultured in the wells of one of the provided 96-well plates, treated as desired and then assayed using the FACE protocol with only the phospho-EGFR antibody. The relative number of cells in each well is then determined through use of the Crystal Violet reagent. In this application, the second 96-well plate can be kept on reserve in case of culturing problems or two 48-well assays can be performed.

FACE EGFR Kits can also be used to determine EGFR phosphorylation relative to the total EGFR protein found in the cells. In this application, the two 96-well plates are cultured as replicates, with the wells within each plate treated with reagents that may affect the phosphorylation state of EGFR. After the cells are fixed, one plate is studied with the phospho-EGFR antibody, while the other plate is studied with the total-EGFR antibody. The relative number of cells in each well is then determined through use of the Crystal Violet reagent. Once the phospho-EGFR and total-EGFR signals have been normalized for cell number, a comparison of the ratio of phosphorylated EGFR to total EGFR for each of the cell growth conditions can be made.

In the FACE EGFR assay, the provided total-EGFR antibody can be used as a positive control to demonstrate that the cells contain EGFR, the kit reagents are functional and that the protocol is performed correctly. Also, because fixed cells are stable for several weeks, you can prepare many plates simultaneously and then perform the FACE assay when desired. Fixed cells should be stored refrigerated in a zip-lock or heat-sealed bag with the formaldehyde solution in the wells.

## Kit Performance and Benefits

**FACE EGFR Kits are for research use only. Not for use in diagnostic procedures.**

**Antibody specificity:** The FACE phospho-EGFR (Tyr992), phospho-EGFR (Tyr1173) and phospho-EGFR (Tyr845) antibodies were raised in rabbits against synthetic phospho-peptides corresponding to residues surrounding phosphorylated Tyr992, Tyr1173 or Tyr845 of human EGF receptor. The phospho-EGFR (Tyr992), phospho-EGFR (Tyr1173) and phospho-EGFR (Tyr845) antibodies recognize EGFR only when phosphorylated at Tyr992, Tyr1173 and Tyr845 respectively. The total-EGFR antibody supplied in the FACE EGFR Kits recognizes EGFR protein regardless of its phosphorylation site.

**Cross-reactivity:** FACE EGFR Kits detect phosphorylated and total EGFR from human, mouse and rat origin.

**Assay time:** < 3 hours of hands-on time.

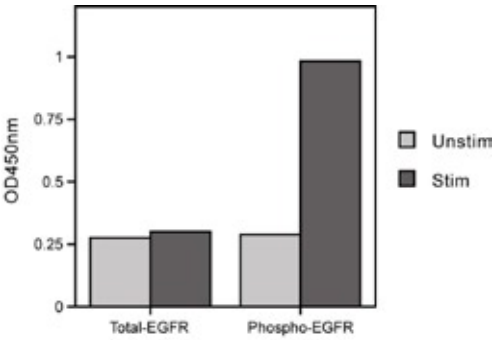



**Figure 1: Measurement of phosphorylated EGFR (Y992) and total EGFR.**
Human A-431 cells were cultured in 96-well plates and serum-starved for 16 hours. Cells were then stimulated with 100 ng/ml amount of Epidermal Growth Factor for 5 minutes and fixed. Total EGFR and EGFR phosphorylated on Tyr992 were each assayed in duplicate using the phospho-EGFR (Y992) and total-EGFR antibodies included in the FACE EGFR (Y992) Kit. Data were plotted after correction for cell number (performed through use of Crystal Violet).

### Note on data interpretation

The phospho-EGFR and total-EGFR antibodies can be used on equivalent cell cultures to determine the effects of various cell treatments on the ratio of phosphorylated EGFR to total EGFR. However, if the signals obtained with the phospho-EGFR antibody and the total-EGFR antibody are identical, one cannot conclude that the treatment resulted in phosphorylation of 100% of the EGFR.

## FACE EGFR Experimental Design

The FACE EGFR assay is a high-throughput method for quantifying cellular levels of EGFR and phosphorylated EGFR. It should be used with cell types that have been shown to contain readily detectable levels of EGFR and, under appropriate induction conditions, phosphorylated EGFR.

Before starting a FACE assay, it is necessary to determine the experimental conditions for each well of the 96-well plate to maximize the information obtained.

**Points to consider:**

1. Are you working with adherent or non-adherent cells? Protocol modifications for use of non-adherent cells are given after the protocol for adherent cells.
2. Do you want to compare phosphorylated EGFR to total EGFR? If so, replicate wells must be cultured so that the two different antibodies can be used on equivalently grown cells.
3 Which wells will be used as positive controls (i.*e.* incubated with the total-EGFR antibody) and which will be used as negative controls (i.*e.* incubated with secondary antibody alone)?
4 Each experimental condition should be performed in duplicate or in triplicate to control for possible errors.
5 FACE assays are most easily performed when all 96 wells of the assay plate are used. This makes it possible to perform washing steps by "flicking" liquid from the plate into a sink. The inverted plate is then tapped gently onto several layers of paper towel to remove the remaining liquid. See "Kit Components" section if you need additional 96-well plates.
6. Fixed cells are stable for several weeks, so you can prepare many plates simultaneously and then perform the FACE assay when desired. Fixed cells should be stored with the formaldehyde solution in the wells and then sealed in a zip-lock bag or, preferably, a heat-sealed bag and refrigerated.

After planning the experiment, determine the amount of each buffer/reagent required and prepare according to the Quick Chart for Preparing Buffers. Multi-channel pipettors and pipettor reservoirs should be used when appropriate. The volumes given are appropriate for multi-channel pipetting if the assay is performed on 48 wells or more. Volumes may need to be adjusted if the assay is performed on less than 48 wells.

## Kit Components and Storage - Colorimetric Assay

FACE Colorimetric Kit components can be stored at -20°C prior to first use. Then, we recommend storing each component at the temperature indicated in the table below.

| Reagents | Quantity<br>1 x 96 rxns / 5 x 96 rxns | Storage / Stability |
|---|---|---|
| Phospho-EGFR antibody | 22 µl / 110 µl | -20°C for 6 months |
| Total-EGFR antibody | 22 µl / 110 µl | 4°C for 6 months |
| Anti-rabbit HRP-conjugated IgG | 22 µl / 110 µl | 4°C for 6 months |
| 1X Antibody Blocking Buffer | 22 ml / 110 ml | -20°C for 6 months |
| 1X Antibody Dilution Buffer | 30 ml / 150 ml | -20°C for 6 months |
| 10X PBS (pH 7.4) | 120 ml / 600 ml | Room temperature for 6 months |
| 10% Triton X-100 | 7 ml / 35 ml | Room temperature for 6 months |
| Crystal Violet Solution | 22 ml / 110 ml | 4°C for 6 months |
| Developing Solution | 22 ml / 110 ml | 4°C for 6 months |
| Stop Solution | 22 ml / 110 ml | 4°C for 6 months |
| 1% SDS Solution | 22 ml / 110 ml | Room temperature for 6 months |
| 96-well tissue culture plate* | 2 / 10 | |
| Plate sealing tape | 2 / 10 | |

* Suitable tissue culture plates are Greiner part no. 655180 and Corning Costar part no. 3596.

### Additional materials required

- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Rocking platform
- Parafilm
- Microplate spectrophotometer capable of reading at 595 nm and at 450 nm (655 as optional reference wavelength)
- Fresh 10% hydrogen peroxide ($H_2O_2$) in $dH_2O$ (3 ml are required)
- 10 mg/ml poly-L-Lysine (if using non-adherent cells)
- 10% Sodium Azide ($NaN_3$) in $dH_2O$ (250 µl are required)
- 37% Formaldehyde (2.5 ml are required for adherent cells; 5.0 ml required for non-adherent cells)

**WARNING:** Sodium Azide and Formaldehyde are highly toxic chemicals. Appropriate safety precautions (gloves and eye protection) should be used. In addition, formaldehyde is highly toxic by inhalation and should be used only in a ventilated hood.

## Kit Components and Storage - Chemiluminescent Assay

FACE Chemi Kit components can be stored at -20°C prior to first use. Then, we recommend storing each component at the temperature indicated in the table below.

| Reagents | Quantity<br>1 x 96 rxns / 5 x 96 rxns | Storage / Stability |
|---|---|---|
| Phospho-EGFR antibody | 11 µl / 55 µl | -20°C for 6 months |
| Total-EGFR antibody | 11 µl / 55 µl | 4°C for 6 months |
| Anti-rabbit HRP-conjugated IgG | 22 µl / 110 µl | 4°C for 6 months |
| 1X Antibody Blocking Buffer | 22 ml / 110 ml | -20°C for 6 months |
| 1X Antibody Dilution Buffer | 30 ml / 150 ml | -20°C for 6 months |
| 10X PBS (pH 7.4) | 120 ml / 600 ml | Room temperature for 6 months |
| 10% Triton X-100 | 7 ml / 35 ml | Room temperature for 6 months |
| Crystal Violet Solution | 22 ml / 110 ml | 4°C for 6 months |
| Chemiluminescent Reagent | 4 ml / 20 ml | 4°C for 6 months |
| Reaction Buffer | 8 ml / 40 ml | 4°C for 6 months |
| 1% SDS Solution | 22 ml / 110 ml | Room temperature for 6 months |
| 96-well tissue culture plate* | 2 / 10 | |
| Plate sealing tape | 2 / 10 | |

* Suitable tissue culture plates are Greiner part no. 655098.

### Additional materials required

- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Rocking platform
- Parafilm
- Microplate spectrophotometer capable of reading at 595 nm for Crystal Violet staining
- Microplate luminometer or CCD camera-coupled imaging system for chemiluminescent detection
- Fresh 10% hydrogen peroxide ($H_2O_2$) in $dH_2O$ (3 ml are required)
- 10 mg/ml poly-L-Lysine (if using non-adherent cells)
- 10% Sodium Azide ($NaN_3$) in $dH_2O$ (250 µl are required)
- 37% Formaldehyde (2.5 ml are required for adherent cells; 5.0 ml required for non-adherent cells)

**WARNING:** Sodium Azide and Formaldehyde are highly toxic chemicals. Appropriate safety precautions (gloves and eye protection) should be used. In addition, formaldehyde is highly toxic by inhalation and should be used only in a ventilated hood.

## Buffer Preparation and Recommendations

We provide an excess of buffer components in order to perform one 96-well FACE assay with the phospho-EGFR antibody and one 96-well FACE assay with the total-EGFR antibody. Required reagents that are not supplied are listed on the previous page. Please review the Quick Chart for Preparing Buffers in this section prior to preparing the assay buffers.

**Preparation of 1X PBS**

1X PBS is the basis of several buffers used in the FACE protocol. 1X PBS is also used in several of the wash steps in the protocol (see the Quick Chart for Preparing Buffers). It is prepared by adding 1 volume of 10X PBS (pH 7.4) to 9 volumes of $dH_2O$ and mixing thoroughly.

**Preparation of Fixing Buffer (4% or 8% Formaldehyde in PBS)**

Fixing Buffer is used to fix cells after cell culturing. It is prepared by adding formaldehyde to 1X PBS and mixing well. 4% formaldehyde is used with adherent cells, 8% formaldehyde is used with non-adherent cells. The recipe in the Quick Chart for Preparing Buffers is written for use with a stock solution of 37% formaldehyde.

**Preparation of Wash Buffer (0.1% Triton X-100 in PBS)**

Wash Buffer is used throughout the FACE protocol and is prepared by adding the provided 10% Triton X-100 solution to 1X PBS and mixing thoroughly.

**Quenching Buffer (Wash Buffer containing 1% $H_2O_2$ and 0.1% Azide)**

Quenching Buffer is used to inactivate the cells' endogenous peroxidase activity. It is prepared by adding fresh Sodium Azide and fresh hydrogen peroxide to the Wash Buffer.

**Blocking Buffer**

This is supplied ready-to-use. A small amount of white precipitate may form if thawed in a warm water bath. This does not interfere with buffer function.

**Antibody Dilution Buffer**

This is supplied ready-to-use. A small amount of white precipitate may form if thawed in a warm water bath. This does not interfere with buffer function.

**Diluted phospho-EGFR antibody**

The phospho-EGFR (Y992) antibody recognizes only the Tyr992 phosphorylated form of the EGFR protein. The phospho-EGFR (Y1173) antibody recognizes only the Tyr1173 phosphorylated form of the EGFR protein. The phospho-EGFR (Y845) antibody recognizes only the Tyr845 phosphorylated form of the EGFR protein. The phospho-antibody will be diluted 1/200 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers in this section).

**Diluted total-EGFR antibody**

The total-EGFR antibody recognizes both the non-phosphorylated and the phosphorylated forms of EGFR protein. The supplied antibody will be diluted 1/200 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers in this section).

**Diluted HRP-conjugated secondary antibody**

HRP-conjugated anti-rabbit IgG is used as the secondary antibody to detect bound primary antibodies. The supplied antibody will be diluted 1/1000 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers in this section).

**1% SDS Solution**

1% SDS Solution is used in the Crystal Violet counting procedure to solubilize cells and release the dye for subsequent quantification at 595 nm. This buffer is supplied ready-to-use.

**Crystal Violet Solution**

This is supplied ready-to-use. Crystal Violet is used to determine the relative number of cells in each well. This stain binds to cell nuclei and gives an $OD_{595}$ reading that is proportional to cell number.

**Developing Solution**

The Developing Solution must be warmed to room temperature before use. This solution is light sensitive, therefore, we recommend avoiding direct exposure to intense light during storage. The Developing Solution may develop a yellow hue over time. This does not affect product performance. A blue color present in the solution indicates that it has been contaminated and must be discarded. Prior to use, transfer the amount of Developing Solution required for the assay into a secondary container (see the Quick Chart for Preparing Buffers in this section), avoid direct exposure to intense light and leave at room temperature for at least 1 hour. After use, discard any remaining solution that was transferred into the secondary container.

**Stop Solution**

Prior to use, transfer the amount of Stop Solution required for the assay into a secondary container (see the Quick Chart for Preparing Buffers in this section). After use, discard any remaining Stop Solution that was transferred into the secondary container.

**WARNING:** The Stop Solution is corrosive. Wear personal protective equipment when handling, *i.e.* labcoat, gloves and eye protection.

## Quick Chart for Preparing Buffers - Colorimetric Assay

| Reagents to prepare | Components | 1 well | 48 wells | 96 wells | 192 wells |
|---|---|---|---|---|---|
| Fixing Buffer for adherent cells | 1X PBS | 98 µl | 4.7 ml | 9.41 ml | 18.82 ml |
| | 37% Formaldehyde | 12 µl | 576 µl | 1.15 ml | 2.30 ml |
| | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Fixing Buffer for non-adherent cells | 1X PBS | 86.0 µl | 4.13 ml | 8.26 ml | 16.51 ml |
| | 37% Formaldehyde | 24.0 µl | 1.15 ml | 2.30 ml | 4.61 ml |
| | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Wash Buffer | 1X PBS | 3.376 ml | 162 ml | 310 ml | 620 ml |
| | 10% Triton X-100 | 34.1 µl | 1.64 ml | 3.13 ml | 6.26 ml |
| | **TOTAL REQUIRED** | **3.41 ml** | **163.7 ml** | **313 ml** | **626 ml** |
| Quenching Buffer | Wash Buffer | 97.9 µl | 4.7 ml | 9.40 ml | 18.8 ml |
| | 10% $H_2O_2$ | 11 µl | 528 µl | 1.06 ml | 2.11 ml |
| | 10% Azide | 1.1 µl | 52.8 µl | 106 µl | 211 µl |
| | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Blocking Buffer | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Diluted total-EGFR antibody | Antibody Dilution Buffer | 45 µl | 2160 µl | 4320 µl | - |
| | Total-EGFR antibody | 0.225 µl | 10.8 µl | 21.6 µl | - |
| | **TOTAL REQUIRED** | **45.22 µl** | **2170.8 µl** | **4341.6 µl** | **-** |
| Diluted phospho-EGFR antibody | Antibody Dilution Buffer | 45 µl | 2160 µl | 4320 µl | - |
| | Phospho-EGFR antibody | 0.225 µl | 10.8 µl | 21.6 µl | - |
| | **TOTAL REQUIRED** | **45.22 µl** | **2170.8 µl** | **4341.6 µl** | **-** |
| Diluted HRP-conjugated secondary antibody | Antibody Dilution Buffer | 110 µl | 5.28 ml | 10.56 ml | 21.12 ml |
| | HRP-conjugated secondary ab | 0.11 µl | 5.28 µl | 10.56 µl | 21.12 µl |
| | **TOTAL REQUIRED** | **110.11 µl** | **5.285 ml** | **10.57 ml** | **21.14 ml** |
| 1X PBS (for wash steps) | 10X PBS | 154 µl | 7.39 ml | 14.11 ml | 28.22 ml |
| | $dH_2O$ | 1.39 ml | 66.53 ml | 127.01 ml | 254.02 ml |
| | **TOTAL REQUIRED** | **1.54 ml** | **73.92 ml** | **141.12 ml** | **282.24 ml** |
| 1% SDS Solution | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Developing Solution | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Stop Solution | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Crystal Violet Solution | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |

## Adherent Cell Protocol - Colorimetric Assay

**PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING**

**Step 1: Culture, fix and block cells**

1. Seed cells in the 96-well plate so that they will be approximately 80% confluent at the time of fixing, after they have been treated as desired. The growth area in each well of the 96-well plate is 0.32 $cm^2$. The provided plates are sterile and treated for tissue culture.
2. Grow and treat cells as desired.
3. Fix cells by replacing the growth medium with 100 µl of 4% formaldehyde in PBS. To minimize the escape of formaldehyde vapors, place a 10 cm x 17 cm piece of parafilm over the plate and then cover the plate with the lid. The covered plate can also be placed in a ziplock bag. Incubate for 20 minutes at room temperature.

   **WARNING:** Formaldehyde is highly toxic. Confine vapors to a chemical hood and wear appropriate gloves and eye protection when using this chemical.

4. Remove formaldehyde solution and wash cells 3 times with 200 µl Wash Buffer. Each wash step should be performed for 5 minutes with gentle shaking.
5. Remove Wash Buffer, add 100 µl Quenching Buffer and incubate for 20 minutes at room temperature.
6. Remove Quenching Buffer and wash cells 2 times for 5 minutes each with 200 µl Wash Buffer.
7. Remove Wash Buffer, add 100 µl Antibody Blocking Buffer and incubate 1 hour at room temperature.

**Step 2: Binding of primary and secondary antibodies**

**NOTE:** Depending on experiment design, some wells may be incubated with diluted phospho-EGFR antibody, some with total-EGFR antibody and some with secondary antibody alone (negative controls). For negative control wells, incubate with 40 µl Antibody Dilution Buffer during primary antibody incubation step.

1. Remove Antibody Blocking Buffer and wash cells 2 times with 200 µl Wash Buffer.
2. Remove Wash Buffer, add 40 µl of diluted primary antibody (or Antibody Dilution Buffer for negative control wells) and seal plate with sealing tape. Place a 10 cm x 17 cm piece of parafilm over the plate, cover with lid and incubate overnight at 4°C. Be sure that the plate is level and that each well is tightly sealed with the sealing tape to prevent evaporation.

   **NOTE:** In cells known to generate high amounts of phosphorylated-EGFR, a three hour primary antibody incubation is sufficient. For maximum sensitivity an overnight incubation is recommended.

3. Remove primary antibody, wash cells 3 times for 5 minutes each with 200 µl Wash Buffer.

4. Remove Wash Buffer, add 100 µl diluted secondary antibody, cover plate with tissue culture plate lid or sealing tape, and incubate 1 hour at room temperature.
5. During this incubation, transfer the amount of Developing Solution required for the assay into a secondary container and leave at room temperature for at least an hour (avoid light).

**Step 3: Colorimetric reaction**

1. Remove secondary antibody, wash cells 3 times for 5 minutes with 200 µl Wash Buffer and then 2 times for 5 minutes with 200 µl 1X PBS.
2. Transfer the amount of Developing Solution required for the assay into a secondary container. Remove PBS from plate wells and add 100 µl Developing Solution to each well.
3. Incubate 2-20 minutes at room temperature protected from direct light. Monitor the blue color development until the darkest-staining wells are medium- to dark-blue. Do not over-develop.
4. Add 100 µl Stop Solution. This acidic solution turns the blue color to yellow. Take care with pipetting to ensure that each well is developed for the same amount of time.

   **WARNING:** The Stop Solution is corrosive. Wear personal protective equipment when handling, *i.e.* labcoat, gloves and eye protection.

5. Read absorbance on a spectrophotometer within 5 minutes at 450 nm with an optional reference wavelength of 655 nm.

**OPTIONAL - Crystal Violet cell staining**

Crystal Violet is an intense stain that binds to the cell nuclei and gives an $OD_{595}$ reading that is proportional to cell number. If you wish to normalize your readings from above simply follow the steps below.

1. After reading at 450 nm is complete, wash wells twice with 200 µl Wash Buffer and 2 times with 200 µl 1X PBS. Tap plates onto paper towels to remove excess liquid from wells and air-dry at room temperature for 5 minutes.
2. Add 100 µl Crystal Violet solution to each well and incubate 30 minutes at room temperature.

   **WARNING:** Crystal Violet is an intense stain. Avoid contact with skin and clothing.

3. Wash wells 3 times with 200 µl 1X PBS for 5 minutes each.
4. Add 100 µl of 1% SDS Solution to each well and incubate on shaker for 1 hour at room temperature.
5. Read absorbance on a spectrophotometer at 595 nm. If the signals obtained are greater than the range of your spectrophotometer, the signal can be reduced by removing some (*e.g.* 50 µl) of the liquid from each well and replacing with an equivalent volume of $dH_2O$.
6. The measured $OD_{450}$ readings are corrected for cell number by dividing the $OD_{450}$ reading for a given well by the $OD_{595}$ reading for that well.

## Non-Adherent Cell Protocol - Colorimetric Assay

The protocol given above can be modified for use with non-adherent cells by culturing and fixing the cells as follows:

1. Treat the 96-well culture plate with 10 µg/ml poly-L-Lysine for 30 minutes at 37°C. Wash twice for 5 minutes with PBS.
2. Seed 17,000 cells/well, or whatever amount is appropriate for your particular cell line.
3. Grow and treat cells as desired.
4. Fix cells by replacing the growth medium with 100 µl of 8% formaldehyde in PBS. Incubate 20 minutes at room temperature.
5. Continue with Step 1, No. 4 of the Adherent Cell Protocol above.

## Buffer Preparation and Recommendations

We provide an excess of buffer components in order to perform one 96-well FACE assay with the phospho-EGFR antibody and one 96-well FACE assay with the total-EGFR antibody. Required reagents that are not supplied are listed on the previous page. Please review the Quick Chart for Preparing Buffers in this section prior to preparing the assay buffers.

**Preparation of 1X PBS**

1X PBS is the basis of several buffers used in the FACE protocol. 1X PBS is also used in several of the wash steps in the protocol (see the Quick Chart for Preparing Buffers). It is prepared by adding 1 volume of 10X PBS (pH 7.4) to 9 volumes of $dH_2O$ and mixing thoroughly.

**Preparation of Fixing Buffer (4% or 8% Formaldehyde in PBS)**

Fixing Buffer is used to fix cells after cell culturing. It is prepared by adding formaldehyde to 1X PBS and mixing well. 4% formaldehyde is used with adherent cells, 8% formaldehyde is used with non-adherent cells. The recipe in the Quick Chart for Preparing Buffers is written for use with a stock solution of 37% formaldehyde.

**Preparation of Wash Buffer (0.1% Triton X-100 in PBS)**

Wash Buffer is used throughout the FACE protocol and is prepared by adding the provided 10% Triton X-100 solution to 1X PBS and mixing thoroughly.

**Quenching Buffer (Wash Buffer containing 1% $H_2O_2$ and 0.1% Azide)**

Quenching Buffer is used to inactivate the cells' endogenous peroxidase activity. It is prepared by adding fresh Sodium Azide and fresh hydrogen peroxide to the Wash Buffer.

**Blocking Buffer**

This is supplied ready-to-use. A small amount of white precipitate may form if thawed in a warm water bath. This does not interfere with buffer function.

**Antibody Dilution Buffer**

This is supplied ready-to-use. A small amount of white precipitate may form if thawed in a warm water bath. This does not interfere with buffer function.

**Diluted phospho-EGFR antibody**

The phospho-EGFR (Y992) antibody recognizes only the Tyr992 phosphorylated form of the EGFR protein. The phospho-EGFR (Y1173) antibody recognizes only the Tyr1173 phosphorylated form of the EGFR protein. The phospho-EGFR (Y845) antibody recognizes only the Tyr845 phosphorylated form of the EGFR protein. The phospho-antibody will be diluted 1/400 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers in this section).

**Diluted total-EGFR antibody**

The total-EGFR antibody recognizes both the non-phosphorylated and the phosphorylated forms of EGFR proteins. The supplied antibody will be diluted 1/400 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers in this section).

**Diluted HRP-conjugated secondary antibody**

HRP-conjugated anti-rabbit IgG is used as the secondary antibody to detect bound primary antibodies. The supplied antibody will be diluted 1/1000 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers in this section).

**Preparation of Chemiluminescent Working Solution**

The Chemiluminescent Reagent and Reaction Buffer should be warmed to room temperature before use. These components are light sensitive, therefore, we recommend avoiding direct exposure to intense light during storage. Prior to use, place the Chemiluminescent Reagent and Reaction Buffer at room temperature for at least 1 hour. In a separate container, mix 1 volume of Chemiluminescent Reagent with 2 volumes of Reaction Buffer to prepare the Chemiluminescent Working Solution (see the Quick Chart for Preparing Buffers in this section). The Chemiluminescent Working Solution is stable for several hours. After the Chemiluminescent Working Solution is aliquoted into the wells, discard the remaining solution.

**1% SDS Solution**

1% SDS Solution is used in the Crystal Violet counting procedure to solubilize cells and release the dye for subsequent quantification at 595 nm. This buffer is supplied ready-to-use.

**Crystal Violet Solution**

This is supplied ready-to-use. Crystal Violet is used to estimate the relative number of cells in each well. This stain binds to cell nuclei and gives an $OD_{595}$ reading that is proportional to cell number.

## Quick Chart for Preparing Buffers - Chemiluminescent Assay

| Reagents to prepare | Components | 1 well | 48 wells | 96 wells | 192 wells |
|---|---|---|---|---|---|
| Fixing Buffer for adherent cells | 1X PBS | 98 µl | 4.7 ml | 9.41 ml | 18.82 ml |
| | 37% Formaldehyde | 12 µl | 576 µl | 1.15 ml | 2.30 ml |
| | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Fixing Buffer for non-adherent cells | 1X PBS | 86.0 µl | 4.13 ml | 8.26 ml | 16.51 ml |
| | 37% Formaldehyde | 24.0 µl | 1.15 ml | 2.30 ml | 4.61 ml |
| | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Wash Buffer | 1X PBS | 3.376 ml | 162 ml | 310 ml | 620 ml |
| | 10% Triton X-100 | 34.1 µl | 1.64 ml | 3.13 ml | 6.26 ml |
| | **TOTAL REQUIRED** | **3.41 ml** | **163.7 ml** | **313 ml** | **626 ml** |
| Quenching Buffer | Wash Buffer | 97.9 µl | 4.7 ml | 9.40 ml | 18.8 ml |
| | 10% $H_2O_2$ | 11 µl | 528 µl | 1.06 ml | 2.11 ml |
| | 10% Azide | 1.1 µl | 52.8 µl | 106 µl | 211 µl |
| | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Blocking Buffer | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Diluted total-EGFR antibody | Antibody Dilution Buffer | 45 µl | 2160 µl | 4320 µl | - |
| | Total-EGFR antibody | 0.1125 µl | 5.4 µl | 10.8 µl | - |
| | **TOTAL REQUIRED** | **45.11 µl** | **2165.4 µl** | **4330.8 µl** | **-** |
| Diluted phospho-EGFR antibody | Antibody Dilution Buffer | 45 µl | 2160 µl | 4320 µl | - |
| | Phospho-EGFR antibody | 0.1125 µl | 5.4 µl | 10.8 µl | - |
| | **TOTAL REQUIRED** | **45.11 µl** | **2165.4 µl** | **4330.8 µl** | **-** |
| Diluted HRP-conjugated secondary antibody | Antibody Dilution Buffer | 110 µl | 5.28 ml | 10.56 ml | 21.12 ml |
| | HRP-conjugated secondary ab | 0.11 µl | 5.28 µl | 10.56 µl | 21.12 µl |
| | **TOTAL REQUIRED** | **110.11 µl** | **5.285 ml** | **10.57 ml** | **21.14 ml** |
| 1X PBS (for wash steps) | 10X PBS | 154 µl | 7.39 ml | 14.11 ml | 28.22 ml |
| | $dH_2O$ | 1.39 ml | 66.53 ml | 127.01 ml | 254.02 ml |
| | **TOTAL REQUIRED** | **1.54 ml** | **73.92 ml** | **141.12 ml** | **282.24 ml** |
| Chemiluminescent Working Solution | Chemiluminescent Reagent | 18 µl | 864 µl | 1.728 ml | 3.46 ml |
| | Reaction Buffer | 36 µl | 1.728 ml | 3.456 ml | 6.91 ml |
| | **TOTAL REQUIRED** | **54 µl** | **2.592 ml** | **5.184 ml** | **10.37 ml** |
| 1% SDS Solution | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |
| Crystal Violet Solution | **TOTAL REQUIRED** | **110 µl** | **5.28 ml** | **10.56 ml** | **21.12 ml** |

## Adherent Cell Protocol - Chemiluminescent Assay

**PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING**

**Step 1: Culture, fix and block cells**

1. Seed cells in the 96-well plate so that they will be approximately 80% confluent at the time of fixing, after they have been treated as desired. The growth area in each well of the 96-well plate is 0.32 $cm^2$. The provided plates are sterile and treated for tissue culture.
2. Grow and treat cells as desired.
3. Fix cells by replacing the growth medium with 100 µl of 4% formaldehyde in PBS. To minimize the escape of formaldehyde vapors, place a 10 cm x 17 cm piece of parafilm over the plate and then cover the plate with the lid. The covered plate can also be placed in a ziplock bag. Incubate for 20 minutes at room temperature.

   **WARNING:** Formaldehyde is highly toxic. Confine vapors to a chemical hood and wear appropriate gloves and eye protection when using this chemical.

4. Remove formaldehyde solution and wash cells 3 times with 200 µl Wash Buffer. Each wash step should be performed for 5 minutes with gentle shaking.
5. Remove Wash Buffer, add 100 µl Quenching Buffer and incubate for 20 minutes at room temperature.
6. Remove Quenching Buffer and wash cells 2 times for 5 minutes each with 200 µl Wash Buffer.
7. Remove Wash Buffer, add 100 µl Antibody Blocking Buffer and incubate 1 hour at room temperature.

**Step 2: Binding of primary and secondary antibodies**

**NOTE:** Depending on experiment design, some wells may be incubated with diluted phospho-EGFR antibody, some with total-EGFR antibody and some with secondary antibody alone (negative controls). For negative control wells, incubate with 40 µl Antibody Dilution Buffer during primary antibody incubation step.

1. Remove Antibody Blocking Buffer and wash cells 2 times with 200 µl Wash Buffer.
2. Remove Wash Buffer, add 40 µl of diluted primary antibody (or Antibody Dilution Buffer for negative control wells) and seal plate with sealing tape. Place a 10 cm x 17 cm piece of parafilm over the plate, cover with lid and incubate overnight at 4°C. Be sure that the plate is level and that each well is tightly sealed with the sealing tape to prevent evaporation.

   **NOTE:** In cells known to generate high amounts of phosphorylated-EGFR, a three hour primary antibody incubation is sufficient. For maximum sensitivity an overnight incubation is recommended.

3. Remove primary antibody, wash cells 3 times for 5 minutes each with 200 µl Wash Buffer.
4. Remove Wash Buffer, add 100 µl diluted secondary antibody, cover plate with tissue culture

plate lid or sealing tape, and incubate 1 hour at room temperature.

5. During this incubation, place the Chemiluminescent Reagent and Reaction Buffer at room temperature.

**Step 3: Chemiluminescent detection**

1. Remove secondary antibody, wash cells 3 times for 5 minutes with 200 µl Wash Buffer and then 2 times for 5 minutes with 200 µl 1X PBS.
2. Remove PBS from plate wells and add 50 µl room temperature Chemiluminescent Working Solution to each well.
3. Read chemiluminescence using a luminometer or CCD camera system. Readings should be taken within 10 minutes to minimize changes in signal intensity.

**OPTIONAL - Crystal Violet cell staining**

Crystal Violet is an intense stain that binds to the cell nuclei and gives an $OD_{595}$ reading that is proportional to cell number. If you wish to normalize your readings from above simply follow the steps below.

1. After reading chemiluminescence, wash wells twice with 200 µl Wash Buffer and 2 times with 200 µl 1X PBS. Tap plates onto paper towels to remove excess liquid from wells and air-dry at room temperature for 5 minutes.
2. Add 100 µl Crystal Violet solution to each well and incubate 30 minutes at room temperature.

   **WARNING:** Crystal Violet is an intense stain. Avoid contact with skin and clothing.

3. Wash wells 3 times with 200 µl 1X PBS for 5 minutes each.
4. Add 100 µl of 1% SDS Solution to each well and incubate on shaker for 1 hour at room temperature.
5. Read absorbance on a spectrophotometer at 595 nm. If the signals obtained are greater than the range of your spectrophotometer, the signal can be reduced by removing some (*e.g.* 50 µl) of the liquid from each well and replacing with an equivalent volume of $dH_2O$.
6. The measured $OD_{595}$ readings indicate the relative number of cells in each well. This relative cell number is then used to normalize each reading from Step 3.

## Non-Adherent Cell Protocol - Chemiluminescent Assay

The protocol given above is suitable for use with non-adherent cells if the cells are cultured and fixed as follows:

1. Treat the 96-well culture plate with 10 µg/ml poly-L-Lysine for 30 minutes at 37°C. Wash twice for 5 minutes with PBS.
2. Seed 17,000 cells/well, or whatever amount is appropriate for your particular cell line.
3. Grow and treat cells as desired.
4. Fix cells by replacing the growth medium with 100 µl of 8% formaldehyde in PBS. Incubate 20 minutes at room temperature.
5. Continue with Step 1, No. 4 of the Adherent Cell Protocol above.

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* The FACE method was developed in the laboratory of Dr. Maikel P. Peppelenbosch, Laboratory for Experimental Internal Medicine, Academic Medical Centre, Amsterdam, The Netherlands. We thank Dr. Henri H. Versteeg and Dr. Peppelenbosch for their assistance in developing the FACE Kits.

# Appendix

## Section A. Troubleshooting Guide

| PROBLEM | POSSIBLE CAUSE | RECOMMENDATION |
|---|---|---|
| No signal or weak signal in wells incubated with either phospho-EGFR antibody or total-EGFR antibody | Omission of key reagent | Check that all reagents have been added in the correct order |
| | Substrate or conjugate is no longer active | Test conjugate and substrate for activity |
| | Enzyme inhibitor present | Sodium azide will inhibit the peroxidase reaction, follow our recommendations to prepare buffers |
| | Plate reader or CCD camera settings not optimal | Verify the wavelength (measurement mode) and filter settings in the plate reader |
| | Developing Solution was cold | Bring Developing Solution to room temperature |
| | Inadequate volume of Developing Solution | Check to make sure that correct volume is delivered by pipette |
| | Cells do not contain detectable levels of phospho EGFR and/or total EGFR | Use Western blotting to confirm that cells contain detectable levels of protein(s) of interest. If you do not require all of the included antibodies for FACE assays, they can be used in Colorimetric Western blotting at a 1:400 dilution for the total and a 1:1000 for the phospho antibody |
| | Insufficient number of cells were plated | Plate cells so that they are 80% confluent at time of fixing |
| | Cells did not adhere correctly to plate | Follow protocol for use of non-adherent cells |
| | Cells are not from correct origin | The phospho and total antibodies detect EGFR from human, mouse and rat. |
| | Excessive washing | Wash steps should be 5 minutes each |
| | Incubation of secondary antibody was too long | Incubate secondary antibody for 1 hour |
| High background in all wells | Developing time too long (Colorimetric Assay) | Stop enzymatic reaction as soon as the positive wells turn medium-dark blue |
| | Measurement time too long (Chemiluminescent Assay) | Reduce integration time or exposure time on luminometer or CCD camera |
| | Concentration of antibodies too high | Perform antibody titration to determine optimal working concentration. Start using 1:200 for the phospho- and the total-antibody and 1:1000 for the secondary antibody. The sensitivity of the assay will be decreased |
| | Inadequate washing | Ensure all wells are filled with Wash Buffer and follow washing recommendations |
| | Inadequate quenching or blocking | Ensure that quenching and blocking steps were performed according to the protocol |

| PROBLEM | POSSIBLE CAUSE | RECOMMENDATION |
|---|---|---|
| Uneven color development | Incomplete washing of wells | Ensure all wells are filled with Wash Buffer and follow washing recommendations |
| | Well cross-contamination | Follow washing recommendations |
| No signal or weak signal in wells incubated with phospho-EGFR antibody | Cell culture conditions did not induce phosphorylation of EGFR | Perform Western blot with phospho-EGFR antibody to confirm that cells contain detectable levels of phosphorylated EGFR |
| Antibody solution evaporates from well during overnight incubation with primary antibody | Sealing tape was incorrectly applied | Ensure that each well is sealed when sealing tape is applied and ensure that the parafilm sheet covers the plate completely before the lid is placed on the plate. The plate can also be placed in a zip-lock or heat-sealed bag |
| Insufficient sensitivity | Antibody concentration incorrect | If the cells studied have very low levels of the protein of interest, the sensitivity of detection may be improved by increasing the concentration of primary antibody used and by minimizing the incubation volume. It is possible to perform the overnight incubation in as little as 25 µl, however, this will make multichannel pipetting difficult and requires the plate be carefully sealed and incubated on a level surface. Alternatively, if the cells have easily detectable levels of the phosphorylated protein and the detection of small changes in phosphorylation is desired, sensitivity of the assay may be improved by decreasing the concentration of the phospho antibody used |
| Poor precision | Cross-well read through | The 96-well plates provided are designed to minimize signal cross-well contamination. If possible, do not use the phospho and total antibodies in adjoining wells. If this is not possible, use the total antibody at a higher dilution |

## Section B. Related Products

| TransAM™ Kits | Unit | Catalog No. |
|---|---|---|
| TransAM™ CREB | 1 x 96 rxns | 42096 |
| | 5 x 96 rxns | 42596 |
| TransAM™ pCREB | 1 x 96 rxns | 43096 |
| | 5 x 96 rxns | 43596 |
| TransAM™ c-Myc | 1 x 96 rxns | 43396 |
| | 5 x 96 rxns | 43896 |
| TransAM™ Elk-1 | 1 x 96 rxns | 44396 |
| | 5 x 96 rxns | 44896 |
| TransAM™ MAPK Family | 2 x 96 rxns | 47296 |
| TransAM™ STAT Family | 2 x 96 rxns | 42296 |
| TransAM™ STAT3 | 1 x 96 rxns | 45196 |
| | 5 x 96 rxns | 45696 |
| TransAM™ IRF Family | 2 x 96 rxns | 45296 |

| Cell-based ELISAs | Unit | Catalog No. |
|---|---|---|
| FACE™ AKT | 1 x 96 rxns | 48120 |
| | 5 x 96 rxns | 48620 |
| FACE™ AKT Chemi | 1 x 96 rxns | 48220 |
| | 5 x 96 rxns | 48720 |
| FACE™ ErbB-2 (Y877) | 1 x 96 rxns | 48130 |
| | 5 x 96 rxns | 48630 |
| FACE™ ErbB-2 (Y877) 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 (Y1248) Chemi | 1 x 96 rxns | 48205 |
| | 5 x 96 rxns | 48705 |
| FACE™ ERK1/2 | 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™ JNK | 1 x 96 rxns | 48110 |
| | 5 x 96 rxns | 48610 |
| FACE™ JNK Chemi | 1 x 96 rxns | 48210 |
| | 5 x 96 rxns | 48710 |
| FACE™ p38 | 1 x 96 rxns | 48100 |
| | 5 x 96 rxns | 48600 |
| FACE™ p38 Chemi | 1 x 96 rxns | 48200 |
| | 5 x 96 rxns | 48700 |

| Sandwich ELISAs | Unit | Catalog No. |
|---|---|---|
| FunctionELISA™ IκBα | 1 x 96 rxns | 48005 |
| | 5 x 96 rxns | 48505 |
| FunctionELISA™ TRAIL | 1 x 96 rxns | 48010 |
| | 5 x 96 rxns | 48510 |
| FunctionELISA™ Cytochrome c | 1 x 96 rxns | 48006 |
| | 5 x 96 rxns | 48506 |

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If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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