

## Certificate of Analysis

### Erk 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) Dual Detect CELISA Assay Kit (Fluorogenic Detection)

Catalog # 17-442

Lot # R0801F009

Expiration 9/30/08

#### Kit Description

**Quantity:** Sufficient reagents for 96 assays per kit.

**Storage:** This kit is shipped at -20°C. Upon receipt, see individual components for storage conditions. The cell culture plate should be stored at room temperature. The 20X TBS (Catalog # 20-190), 20% Tween<sup>®</sup> 20 (Catalog # 20-246), 10% BSA (Catalog # 20-191) and Substrate Diluent (Catalog # CS200690) should be stored at 4°C. All other components are stored at -20°C. Aliquot the -20°C components, if necessary, to avoid freeze-thaw cycles.

**Stability:** Kit components are stable for a minimum of 3 months from date of shipment if stored and handled correctly.

#### Kit Components

##### **Black Clear 96-well Cell culture plates**

Catalog # CS201104

One 96-well cell culture plate with clear (view) bottom.

Store at room temperature.

##### **20% Tween<sup>®</sup>-20 (v/v),**

Catalog # 20-246.

One bottle containing 3ml of 20% Tween<sup>®</sup>-20 in sterile MilliQ water. Store at 4°C.

##### **20X TBS**

Catalog # 20-190.

One bottle containing 50 mL of 1M Tris and 3M NaCl, pH 7.4. Store at 4°C.

##### **10% BSA in TBS (Blocking Buffer)**

Catalog # 20-191

One bottle containing 10 mL 10% BSA in 1X TBS.

Store at 4°C.

##### **Substrate Diluent**

Catalog # CS200690

One bottle containing 15 mL of a proprietary buffer.

Store at 4°C.

##### **500X anti-Erk 1/2 (Total) Antibody**

Catalog # CS200691

One vial containing 15 µL of 500X total Erk 1/2 antibody. Store at -20°C.

##### **500X anti-phospho Erk 1/2**

**(Thr202/Tyr204)/(Thr185/Tyr187) Antibody**

Catalog # CS200692

One vial containing 15 µL of 500X phospho Erk 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) antibody.

Store at -20°C.

##### **500X Goat anti-Mouse HRP Detection Reagent**

Catalog # CS200689

One vial containing 50µL of 500X Goat anti-Mouse HRP Detection Reagent. Store at -20°C.

##### **500X Goat anti-Rabbit AP Detection Reagent**

Catalog # CS200674

One vial containing 50µL of 500X Goat anti-Rabbit AP Detection Reagent. Store at -20°C.

Store at -20°C.

##### **200X HRP Substrate**

Catalog # CS200673

One vial containing 60 µL of 200X HRP substrate. Store at -20°C.

##### **200X AP Substrate**

Catalog # CS200701

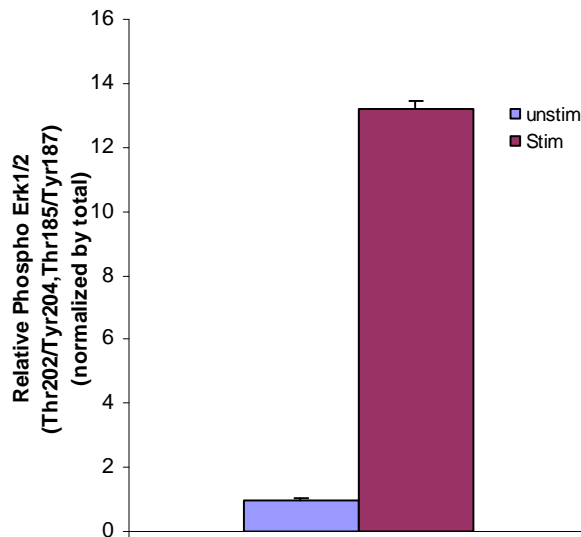
One vial containing 60 µL of 200X AP substrate. Store at -20°C.

**FOR RESEARCH USE ONLY.  
NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS.  
DO NOT USE IN HUMANS.**

**Use:** Dual-Detect CELISA is a simple and efficient cell-based ELISA assay to measure the relative expression levels of both the total and the phosphorylated form of the target in whole cells in a 96 well microplate format using dual fluorescent detection. This allows for the normalization of total protein between wells, thus allowing a more accurate measurement of phosphorylation levels among cell and/or growth condition differentials. Cells are cultured directly in the microplate and stimulated as desired. They are then fixed and simultaneously incubated with two primary antibodies. One is a phosphospecific antibody directed against the phosphorylated epitope that correlates with its activation and the other is a total antibody that recognizes the target-protein, regardless of phosphorylation status. Both the total target protein and the phosphorylated protein are measured in a single well using a double immunoenzymatic labeling procedure, using both horseradish-peroxidase (HRP) and alkaline phosphatase (AP), and two spectrally distinct fluorogenic substrates for HRP and AP. The fluorescence of the phosphorylated protein is normalized to the total protein in each well thus reducing well-to-well variations.

Please refer to the User Manual for further information and a detailed assay procedure.

### Quality Control Testing



**Fig 1. Phospho Erk1/2 (Thr202/Tyr204)(Thr185/Tyr187) Dual-Detect CELISA**

NIH3T3 cells were seeded at  $1 \times 10^4$  cells per well, cultured for 24 hr, serum starved 16 hr, and stimulated with 50ng/mL of PDGF for 5 minutes. Total and phospho Erk1/2 levels were determined using Erk (Thr202/Tyr204)/(Thr185/Tyr187) Dual Detect CELISA Kit. Phosphorylated Erk1/2 levels have been normalized to the total Erk.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.