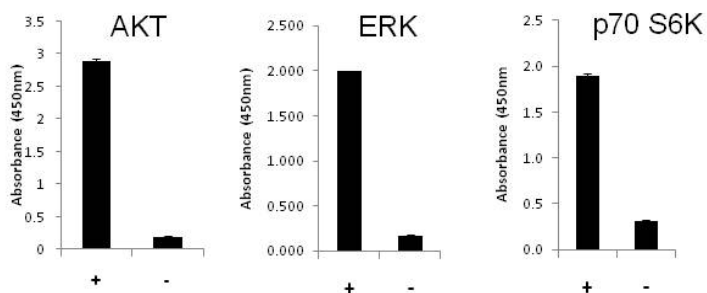


ERK/AKT/p70 S6K Activation InstantOne ELISA™ Kit

Catalog Number: 85-86018

Also known as: N/A

RUO: For Research Use Only. Not for use in diagnostic procedures.



Middle: A431 cells were pre-treated with 10 μ M AG1478 (an EGFR kinase inhibitor), washed and unstimulated (-) or stimulated with 10 ng/mL EGF for 10 min (+). Cells were analyzed for phosphorylated ERK 1/2/3 by InstantOne ELISA™. Left and Right: MCF7 cells were pre-treated with 10 μ M wortmannin washed and unstimulated (-) or stimulated with 10 μ g/mL insulin for 10 min (+). Cells were analyzed for phosphorylated AKT 1/2/3 (left) and p70 S6K (T389) (right) by InstantOne ELISA™.

Product Information

Contents: ERK/AKT/p70 S6K Activation InstantOne ELISA™



Catalog Number: 85-86018



Temperature Limitation: Store at 2-8°C.



Batch Code: Refer to vial



Use By: Refer to vial

Description

The InstantOne ELISA™ is specifically engineered for accurate measurement of phosphorylated ERK 1/2 (human, mouse, hamster), AKT 1/2/3 (human, mouse, hamster), and p70 S6K (human, mouse) in cell lysates. Detection of these phosphorylated molecules in rat is also expected. The InstantOne ELISA™ kit allows for fast analysis of samples in approximately one hour. All reagents used in a traditional sandwich ELISA are added in solution to a plate followed by a wash step and detection with the TMB colorimetric substrate.

Extracellular signal-regulated kinase (ERK) is the founding member and a key component of the classical Mitogen-Activated Protein Kinase (MAPK) pathway. MEK activates ERK 1/2 by phosphorylation of its TxY motifs in response to a variety of stimuli, including growth factors and cytokines.

AKT, which exists as multiple isoforms, is one of the principle kinases activated by phosphoinositide 3-kinase (PI3K). These lipid second messengers bind to the pleckstrin homology domain of AKT to promote its translocation to the plasma membrane for activation via phosphorylation at Thr308 and Ser473 by PDK1 and the mTOR TORC2 complex, respectively. Phosphorylation at both these sites is required for full activation of AKT Ser/Thr kinase activity. p70 S6 Kinase (p70S6K) is a member of the ribosomal S6 kinase family of Ser/Thr kinases. p70 S6K activity is controlled by multiple signaling pathways, including the MAPK, phosphoinositide-3 kinase (PI3K), and mTOR pathways. This regulation results in the phosphorylation of multiple sites of p70 S6K, including Thr389 within the linker domain, which is required for full activation. Activated p70 S6K phosphorylates several residues on the S6 ribosomal protein, thereby leading to increased protein synthesis.

This kit has been developed to recognize the following phosphorylation sites:

ERK 1/2/3 (Thr202/Tyr204, Thr185/Tyr187)

AKT 1/2/3 (Ser473)

p70 S6K (Thr389)

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Components

User Manual

phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) Capture Antibody Reagent (Part No. IOPERKCAR1T) - 1 mL
phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) Detection Antibody Reagent (Part No. IOPERKDAR1T) - 1 mL
phospho-AKT 1/2/3 (Ser437) Capture Antibody Reagent (Part No. IOPAKTACAR1T) - 1 mL
phospho-AKT 1/2/3 (Ser437) Detection Antibody Reagent (Part No. IOPAKTADAR1T) - 1 mL
phospho-p70 S6K (Thr389) Capture Antibody Reagent (Part No. IOPS6KCAR1T) - 1 mL
phospho-p70 S6K (Thr389) Detection Antibody Reagent (Part No. IOPS6KDAR1T) - 1 mL
InstantOne™ Assay Plate (Part No. IOAP96) - 96 well (sealed)
Cell Lysis Buffer (5X) (Part No. IOCLB1) - 12 mL
Enhancer Solution (Part No. IOES1) - 1 mL
Wash Buffer (10X) (Part No. IOWB1) - 15 mL
Detection Reagent (TMB Substrate) (Part No. IODR1) - 12 mL
Stop Solution (Part No. IOSS1) - 12 mL
Positive Control Cell Lysate (lyophilized) (Part No. IOMEAPPC1) – 0.25 mL
Plate seal - 1 ea

Applications Tested

This assay has been validated for the detection of phosphorylated human ERK 1/2 in stimulated A431 cell lysates. In addition, this kit has been validated for the detection of phosphorylated human AKT 1/2/3 and p70 S6K in stimulated MCF7 cell lysates. This assay was evaluated for specificity by immunoblot analysis using the same lysates and antibody.

Related Products

85-86011 ERK 1/2 (Total) InstantOne ELISA™
85-86012 phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) InstantOne ELISA™
85-86047 AKT 1/2/3 (Total) InstantOne ELISA™
85-86042 phospho-AKT 1/2/3 (Ser473) InstantOne ELISA™
85-86051 p70 S6K (Total) InstantOne ELISA™
85-86052 phospho-p70 S6K (Thr389) InstantOne ELISA™
IOCLB InstantOne ELISA™ Cell Lysis Buffer (5X) – 100 mL
IOES InstantOne ELISA™ Enhancer Solution – 100 mL
IOWB InstantOne ELISA™ Wash Buffer(10X) – 500 mL