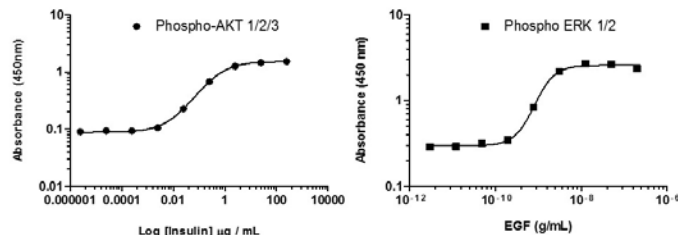


AKT/ERK Activation InstantOne ELISA™ Kit

Catalog Number: 85-86014

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



Left: Serum-starved MCF-7 cells were treated (30 min) with a dose-range of insulin before cell lysis and analysis of AKT 1/2/3 (pS473) phosphorylation using the InstantOne ELISA™. Right: Confluent HeLa cells were serum starved overnight, then stimulated with EGF for 10 min at the indicated concentrations. Cells were analyzed for ERK 1/2 (pT202/Tyr204) phosphorylation using the InstantOne ELISA™.

Product Information

Contents: AKT/ERK Activation InstantOne ELISA™

Catalog Number: 85-86014



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 human, mouse, and hamster ERK 1/2 and AKT 1/2/3 in cell lysates. Detection of rat ERK 1/2 and AKT 1/2/3 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. ERK1 (p44, MAPK3) and ERK2 (p42, MAPK2) are both activated via the MAPK/ERK pathway, which is downstream of various Receptor Tyrosine Kinases (RTKs) or GPCRs. MEK, a Ser/Thr and Tyr kinase, activates ERK by phosphorylation of its TxY motifs, namely Thr202/Tyr204 and Thr185/Tyr187 of ERK1 and ERK2, respectively. Once activated, ERK phosphorylates a variety of proteins that regulate cellular processes such as cell division, proliferation, survival, differentiation, apoptosis, motility, and metabolism.

AKT, which exists as multiple isoforms, is one of the principle kinases activated by phosphoinositide 3-kinase (PI3K). Activation of PI3K results in the generation of phosphatidylinositol (3,4,5)-triphosphate (PIP3). 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. AKT phosphorylates over 50 known substrates, including GSK3, AS160, PRAS40, TSC 1, TSC 2, Raf-1, Bad, the FOXO family of transcription factors, and PFK2. Due to its established critical role in numerous biological processes, AKT is a frequent target for oncology-related drug development.

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

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

AKT 1/2/3 (Ser473)

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Components

User Manual

phospho-AKT 1/2/3 (Ser437) Capture Antibody Reagent (Part No. IOPAKTACAR1M) - 1 x 1.5 mL

phospho-AKT 1/2/3 (Ser437) Detection Antibody Reagent (Part No. IOPAKTADAR1M) - 1 x 1.5 mL

phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) Capture Antibody Reagent (Part No. IOPERKCAR1M) - 1 x 1.5 mL

phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) Detection Antibody Reagent (Part No. IOPERKDAR1M) - 1 x 1.5 mL

InstantOne ELISA™ 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. IOMAEPC1) – 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 and phosphorylated human AKT 1/2/3 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™

85-86042 phospho-AKT 1/2/3 (Ser473) InstantOne ELISA™

85-86047 AKT 1/2/3 (Total) InstantOne ELISA™

IOCLB InstantOne ELISA Cell Lysis Buffer (5X) - 100mL

IOES InstantOne ELISA Enhancer Solution – 100mL

IOWB InstantOne ELISA Wash Buffer(10X) – 500mL