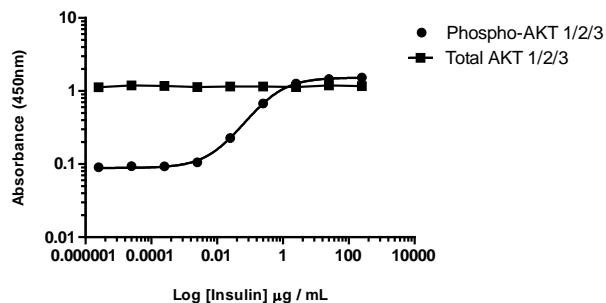


## AKT 1/2/3 (Total/Phospho) InstantOne ELISA™ Kit

**Catalog Number:** 85-86046

**Also known as:** AKT, Protein Kinase B, PKB, RAC-alpha serine/threonine-protein kinase

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



Induction of AKT 1/2/3 (pS473) phosphorylation in MCF-7 cells in response to insulin treatment was analysed by InstantOne ELISA™. 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) and total AKT1/2/3 protein levels using the (Total/Phospho) InstantOne ELISA™.

### Product Information

**Contents:** AKT 1/2/3 (Total/Phospho) InstantOne ELISA™

**Catalog Number:** 85-86046



**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 human, mouse, and hamster total AKT 1/2/3 and phosphorylated AKT 1/2/3 (Ser473) in cell lysates. Detection of rat AKT 1/2/3 is 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.

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.

### Components

This (Total/Phospho) kit includes sufficient reagents to perform 48 wells of the total and 48 wells of the phospho-specific detection for a total of 96 wells.

### User Manual

AKT 1/2/3 (Total) Capture Antibody Reagent (Part No. IOAKTBCAR1M) – 1 x 1.5 mL  
 AKT 1/2/3 (Total) Detection Antibody Reagent (Part No. IOAKTBDAR1M) – 1 x 1.5 mL  
 phospho-AKT 1/2/3 (Ser473) Capture Antibody Reagent (Part No. IOPAKTACAR1M) – 1 x 1.5 mL  
 phospho-AKT 1/2/3 (Ser473) Detection Antibody Reagent (Part No. IOPAKTADAR1M) – 1 x 1.5 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. IOAKTAPC1) – 0.25 mL  
 Plate seal - 1 ea

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### **Applications Tested**

This assay has been validated for the detection of human total AKT 1 and phosphorylated human AKT 1/2/3 (Ser473) in stimulated MCF7 cell lysates. This assay was evaluated for specificity by immunoblot analysis using the same lysates and antibody.

### **Related Products**

85-86014 AKT/ERK Activation InstantOne ELISA™

85-86018 ERK/AKT/p70 S6K Activation 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) – 100 mL

IOES InstantOne ELISA Enhancer Solution – 100 mL

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