



## PhosphoQuest™ cell based ELISA kinase assay with Mithras LB 940

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### Introduction

Kinases are most routinely assayed by using purified kinases, but many applications demand the ability to examine the kinase activity within a biological system. AKT, also known as the protein kinase B- $\alpha$  (PKB- $\alpha$ ) or RAC-PK $\alpha$ , was initially identified as one of the downstream targets of PI-3 Kinase (PI3-K). Activated PI3-K generates 3' phosphoinositide products, 3,4,5-triphosphates (PI-3,4,5-P3) and PI-3,4-P2. AKT is recruited from the cytosol to the plasma membrane through the interaction of its PH domain with these phosphoinositides. Upon membrane localization, AKT undergoes a conformational change, which makes it accessible to phosphorylation at threonine-308 and serine-473 in the kinase domain by PDK-1 and related kinases.



**Figure 1: PhosphoQuest™ kit contents**

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## detect and identify

The DiscoverX AKT1 (Total) ELISA is designed to detect and quantify the levels of AKT1 protein, independent of its phosphorylation state. Although performance characterization of this ELISA kit was done primarily on human cell lines, cross-reactivity of this kit with mouse and rat cells was observed. This assay is intended to detect AKT1 from lysates of cells and can be used to normalize the AKT1 content of the samples when examining quantities of phosphorylated sites on AKT1 using other DiscoverX kits.



**Figure 2: Mithras LB 940 multimode reader**

The Mithras LB 940 is a multimode plate reader with a unique optical design (DOPS – Dedicated Optical Path System) to ensure optimized performance for the detection technologies implied. These are

- luminescence
- BRET/BRET<sup>2</sup>
- fluorescence
- UV/VIS absorbance
- fluorescence polarization
- AlphaScreen™
- TRF
- HTRF®

In addition accessory options, e.g. reagent injectors, temperature control and cooled PMT detection units are available. The possibility to equip the Mithras with up to 4 reagent injectors enables the automation of reagent addition (e.g. stop solution) in the instrument.

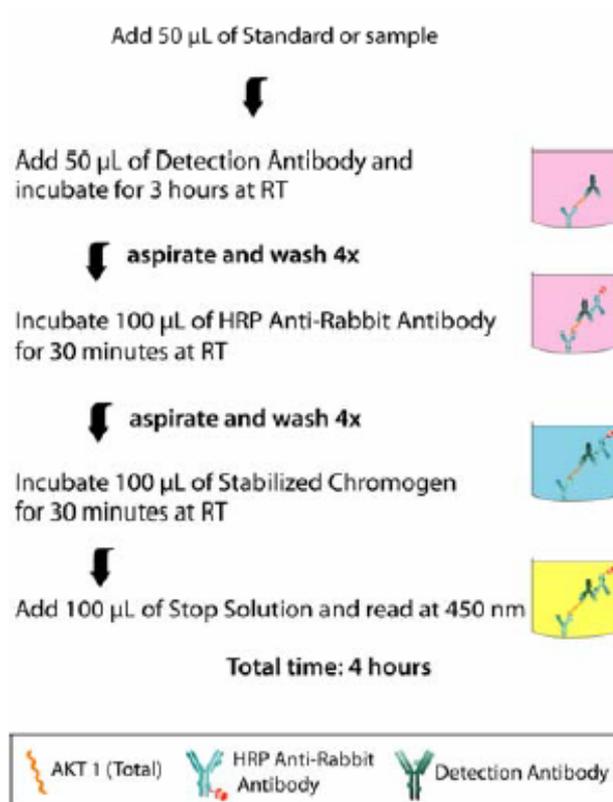


## detect and identify

### Methods

#### Assay Protocol:

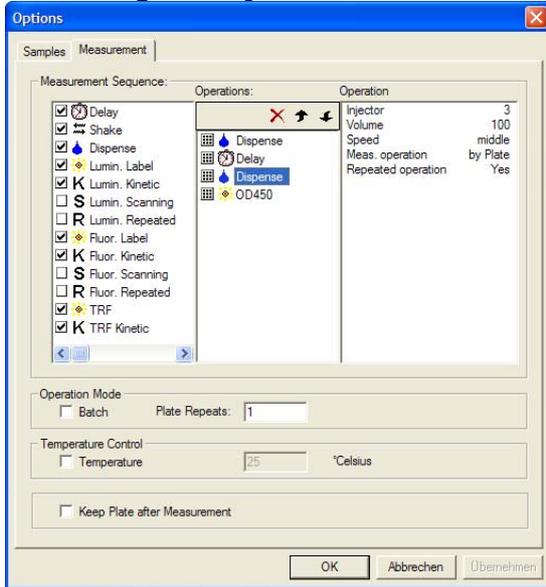
- Add 50  $\mu$ L AKT1 standard to coated plates
- Add 50  $\mu$ L detection antibody
- Incubate 3 hours at room temperature
- Wash plate thoroughly
- Add 100  $\mu$ L anti-rabbit IgG-HRP working solution
- Incubate 30 minutes at room temperature
- Wash plate thoroughly
- Add 100  $\mu$ L chromogen
- Incubate 30 minutes at room temperature
- Add 100  $\mu$ L stop solution
- Read signal in absorbance mode at 450 nm



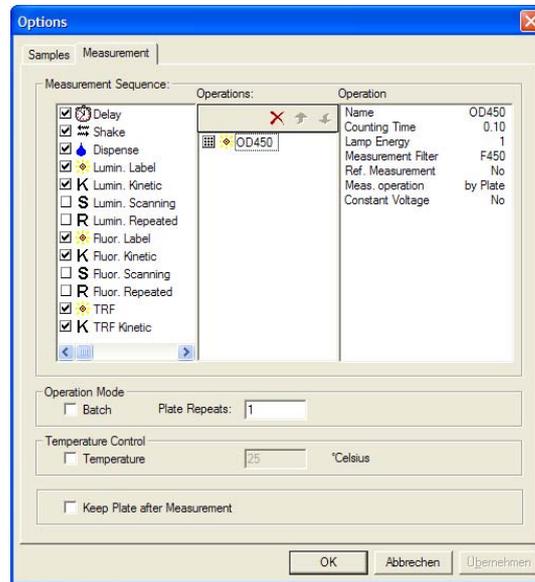


detect and identify

**Instrument settings:**  
*with reagent injectors*



*without reagent injectors*

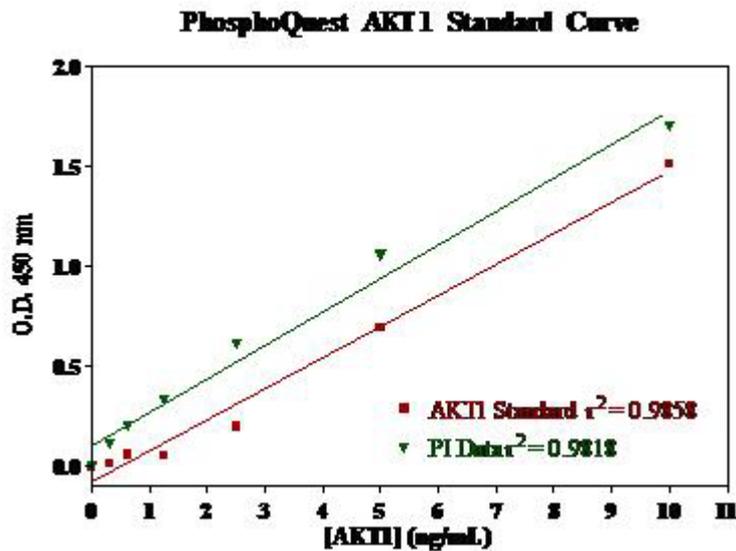




## detect and identify

### Results

A linear response of signal over the entire concentration range from 0 to 10 ng/mL AKT1 can be observed corresponding to the expected values provided in the kit insert.



**Figure 3: plot of OD vs. AKT1 concentration**

### Conclusion

- The PhosphoQuest™ assay provides the ability to detect phosphorylation of specific proteins from cell lysates.
- Linear increase in signal is observed over 2 orders of magnitude within a concentration range between 0 and 10 ng/mL AKT1.



## detect and identify

### **Materials**

- Mithras LB 940, equipped with two reagent injectors and absorbance functionality (Berthold 38099)
- absorbance filter 450 nm (standard in Mithras with absorbance functionality)
- PhosphoQuest™ AKT1 kit (DiscoverX 94-0013)
- additional reagents see kit insert

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