

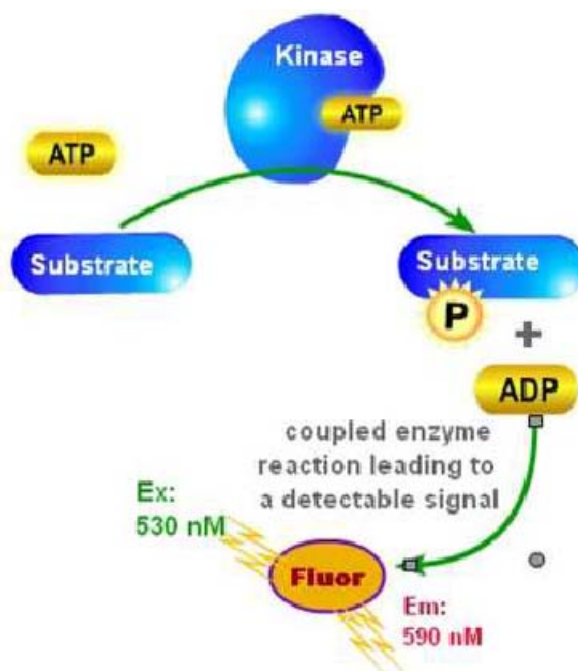


## ADP Quest™ HS kinetic assay with Mithras LB 940

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### Introduction

ADP Quest™ HS is a homogeneous non-antibody assay for the measurement of ADP, a universal product of kinase activity. Unlike alternative generic approaches that monitor the depletion of ATP from a kinase reaction, this method follows a product of the reaction, and offers a convenient gain-of-signal assay format. This kinase assay is a non-radioactive method that does not require use of an antibody or modified substrate and are compatible with both peptides and whole protein substrates.



**Figure 1: ADP Quest HS principle**

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## detect and identify

The ADP Quest HS assay uses a coupled enzyme reaction system to generate hydrogen peroxide from ADP. Hydrogen peroxide when combined with Acetyl Dihydroxy Phenoxazine (ADHP) (fluorescent dye precursor) in the presence of peroxidase generates the fluorescently active resorufin dye. The resorufin fluorescence can be detected on any standard instrument capable of fluorescence excitation and emission and the assay has been optimized to allow for automation.

In Kinetic mode, the detection reagents are added with the kinase reaction components to allow for continuous monitoring of enzyme activity.

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. Especially the fact that at least one injector is located in the reading position fast reaction kinetics can be monitored.



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



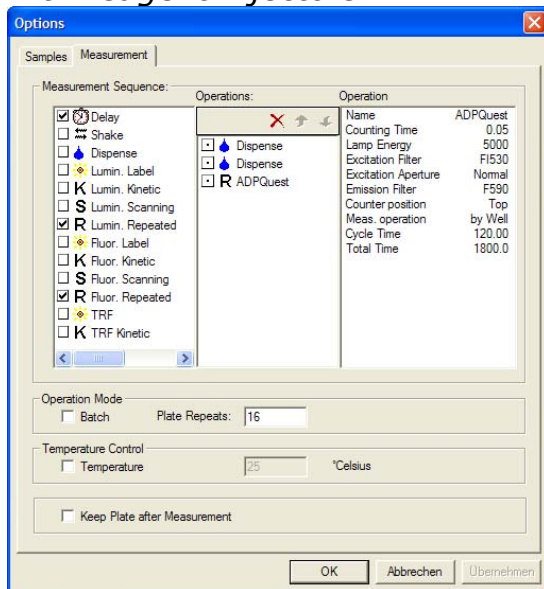
## detect and identify

### Methods

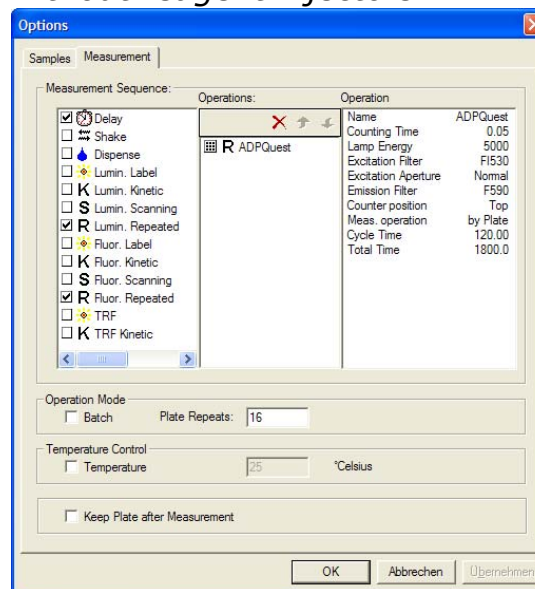
#### **Assay Protocol:**

- 5  $\mu$ L 100  $\mu$ L Kemptide substrate
- 10  $\mu$ L serially diluted kinase
- 10  $\mu$ L ADP Reagent A
- 20  $\mu$ L Reagent B
- 5  $\mu$ L 100  $\mu$ L ATP
- Read every 120 seconds

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



#### without reagent injectors

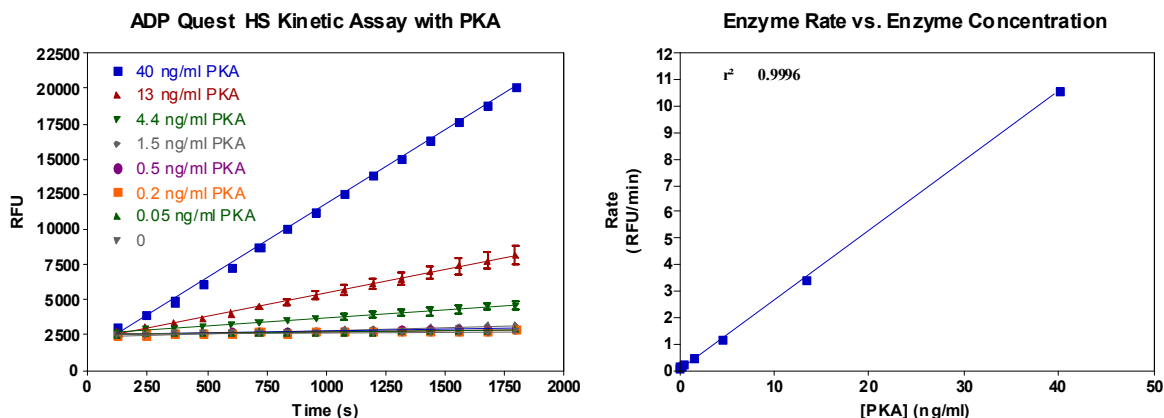




## detect and identify

### Results

A linear increase of signal can be observed over the course of 30 minutes. When plotting the linear rate against enzyme concentration a linear relationship over 2 orders of magnitude can be observed.



**Figure 3 left: Real-time Kinetics.**

**ADP Quest HS assay was run according to the product insert using PKA enzyme at concentrations ranging from 0.05 - 40 ng/mL; signal was read over a period of time resulting in linear increases in signal**

**Figure 3 right: Standard curve**

### Conclusion

- The ADP Quest HS assay is ideal for measuring kinase kinetic activity in continuous mode
- Linear increase in signal observed over the course of 30 minutes
- Linear rate was plotted against enzyme concentration to determine assay dynamic range
- A linear relationship was observed over 2 orders of magnitude
- This indicates excellent performance in measure kinase activity in kinetic mode



## detect and identify

### **Materials**

- Mithras LB 940, equipped with two reagent injectors (Berthold 38099)
- Excitation filter 530 nm (Berthold 37996)
- Emission filter 590 nm (Berthold 37989) or 600 nm (Berthold 40095)
- ADP Quest HS kit (DiscoverX 90-0087)
- Black microplates: 96 well (Berthold 23302) or 384 well
- additional reagents see kit insert

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AlphaScreen™ is a trademark of PerkinElmer  
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