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Application Note

Invitrogen's Adapta™ Universal Kinase Assay using BioTek's Synergy™ 4 Hybrid Microplate Reader

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Considerable interest has been shown in kinases as drug targets because of their link to the regulation of important cellular processes, and various biochemical assays have been developed to measure kinase activity used in high-throughput chemical library screens. Here we describe the use of the Synergy™ 4 Multi-Mode Microplate Reader to measure the Adapta™ Universal Kinase Assay, an assay suitable for all kinases, including difficult to assay targets, such as lipid kinases.

Introduction

The Adapta™ Universal Kinase Assay is a homogeneous, fluorescence-based immunoassay for the detection of ADP. Adapta™ technology uses time-resolved fluorescence resonance energy transfer (TR-FRET), which is a well-recognized technique to reduce background fluorescence. TR-FRET works on the same principles as standard FRET in that when suitable pairs of fluorophores are in close proximity of one another, excitation of the donor fluorophore results in energy transfer to the acceptor fluorophore. The significant difference is the long fluorescence lifetime of the lanthanide donor fluor. This allows measurement of acceptor fluorescence (FRET signal) long after the background fluorescence has dissipated.

The Adapta™ Universal Kinase Assay can be divided into two phases. The first phase is the kinase reaction, while the second is the ADP detection (Figure 1). All the constituents for the kinase reaction are added to the well and the reaction allowed to occur. Detection occurs afterward by the addition of Europium-labeled anti-ADP antibody, an Alexa Fluor® 647 labeled ADP tracer, and EDTA to stop the kinase reaction. ADP formed as a result of the kinase reaction (without inhibitor) will displace the Alexa Fluor® 647 labeled ADP tracer, resulting in a TR-FRET signal decrease. In the presence of an inhibitor, the amount of ADP formed by the kinase reaction is reduced, and the resulting intact antibody-tracer interaction results in a high TR-FRET signal.

Materials and Methods

Using the Adapta™ Universal Kinase Assay Kit (PV5099) from Invitrogen (Carlsbad, CA), a series of ATP/ADP mixtures ranging from 100% to 0.01% ADP was prepared such that the total nucleoside concentration remained constant. The ATP/ADP mixtures were aliquoted in 10µL replicates of 3 into wells of a solid white 384-well microplate. This served to mimic the kinase reaction, which normally would be allowed to incubate for 60 minutes at room temperature. A 3x detection solution was prepared consisting of 30 mM EDTA, 6 nM Eu-labeled Anti-ADP antibody and 3x Alexa Fluor® 647 ADP tracer in TR-FRET dilution buffer. After incubation, 5 µL of the 3x detection solution was added to all the wells and allowed to equilibrate for 30 minutes at room temperature. After incubation with the detection solution, the fluorescence of each sample was measured.

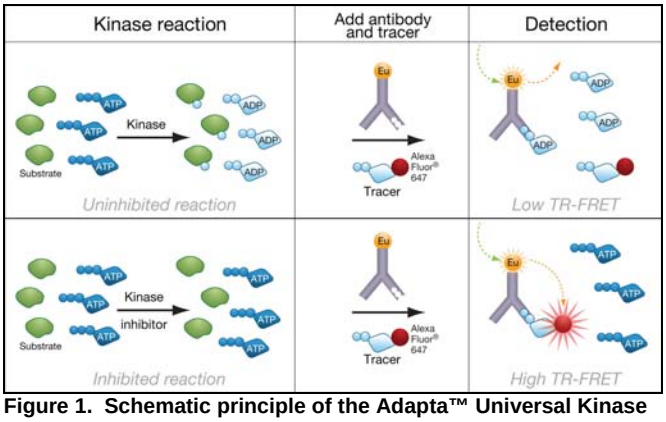

Figure 1. Schematic principle of the Adapta™ Universal Kinase Assay.

Time-resolved fluorescence measurements were made using a Synergy™ 4 Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT). Samples were measured with a high intensity xenon flash lamp using a 340/30-excitation filter with both a 620/10 and a 665/8 emission filters to measure Europium and Alexa Fluor® 647 TR-FRET emissions respectively. Light was directed using a 400 nm cut off dichroic mirror and the signal detected after a 100 µsec delay, with a collection time of 200 µsec and a PMT sensitivity setting of 135 for both filter sets.

The Synergy 4 reader was controlled and the data collected and analyzed using Gen5™ Data Analysis Software (BioTek Instruments).

Results

The data presented in Figure 2 demonstrates the ability of the Synergy 4 Multi-Mode Microplate Reader to measure the conversion of ATP to ADP by kinase reactions. The ratio of the 665 nm to 620 nm signal decreases 10-fold with increasing amounts of conversion, and analysis at 100% and 20% conversion relative to 0% conversion demonstrates an excellent Z'-factor of 0.8.

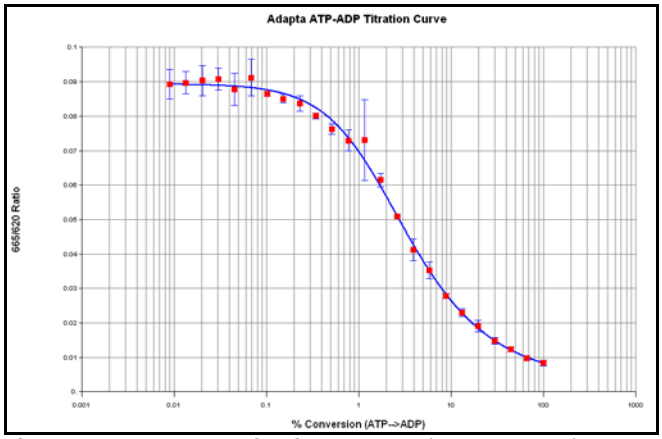

Figure 2. ATP to ADP Titration Curve. The 665 nm and 620 nm signals were obtained and the 665/620-ratio calculated using Gen5 and plotted as a function of the percent conversion to ADP. Data points and error bars represent the mean and standard deviation of 3 data points each.

Discussion

This data demonstrates the ability of the Synergy™ 2 and Synergy™ 4 Multi-Mode Microplate Readers to measure the TR-FRET signal required for robust measurements of the Adapta™ Universal Kinase Assay. The Synergy readers, in conjunction with Gen5 Data Analysis Software, provide a high degree of sensitivity along with easy data interpretation and analysis in one package.

The Adapta™ Universal Kinase Assay measures kinase activity by detecting ADP formation. Most of the signal change occurs in the first 10-20% conversion of ATP to ADP (Figure 2). This is in sharp contrast to kinase assays that measure ATP depletion, in which 20% conversion of ATP to ADP results in only a 20% signal change. As a result, the Adapta™ Universal Kinase Assays produces high Z'-factors at low % conversions and is ideally suited for use with kinases with low activity since less ADP has to be formed to achieve an optimal assay window.

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