



Omnia[®] Kinase Assay from Invitrogen[™] on Synergy[™] 4

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Introduction

Here we describe the use of the Synergy 4 Hybrid Microplate Reader to measure the output from Invitrogen's Omnia[®] kinase assay technology.

Omnia[®] kinase assays utilize a fluorescence peptide substrate-based technology for the rapid, homogenous and sensitive real-time detection of kinetic enzymatic activity. The chelation-enhanced fluorophore (CHEF), also referred to as Sox, is an unnatural amino acid that can be incorporated into substrate peptides using standard solid-phase peptide chemistry. Upon phosphorylation by a kinase, a magnesium ion is chelated to form a bridge between the Sox moiety and the phosphate group (Figure 1). The presence of the magnesium chelate bridge results in an increase in fluorescence when the compound is excited at 360 nm and the emission measured at 485 nm.

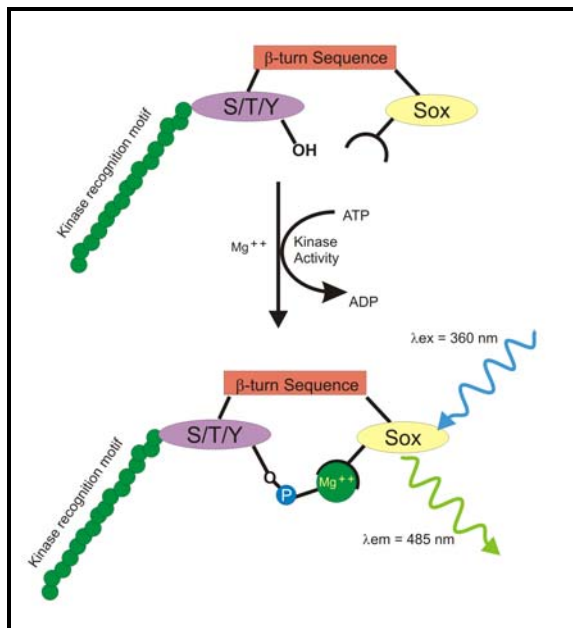


Figure 1. Schematic diagram of the Omnia[®] Kinase Assay.

Materials and Methods

An Omnia[®] Tyrosine Kinase assay kit, catalog number KNZ4031, and Syk kinase enzyme, catalog number PV3857, were obtained from Invitrogen (Carlsbad, CA). Solid white 384-well microplates, catalog number 3705, were purchased from Corning (Corning, NY).

The assay was carried out according to the kit instructions. Assays were performed such that either the amount of kinase or the amount of substrate varied from well to well. For assays that utilized constant amounts of kinase, serial dilutions of the Tyr Peptide 3 substrate were made using deionized water as the diluent. 5 μ L of each Peptide substrate dilution were pipetted in replicates of 8 or 16 into wells of a white 384-well microplate. Reactions were initiated by the addition of 45 μ L of master mix. The master mix was made such that in a final reaction volume of 50 μ L the solution contained 1 X proprietary reaction buffer, 1 mM ATP, 1 mM DTT and 10 ng of Syk kinase.

To assess the affect of kinase concentrations on activity, dilutions of Syk kinase were made using deionized water as the diluent. Aliquots (5 μ L) containing 40, 20, 10, or 0 ng of Syk kinase were pipetted into white 384-well microplates. A similar master mix was prepared except that the kinase enzyme was replaced with Tyr peptide 3 substrate (5 μ M final). Reactions were initiated by the addition of 45 μ L of master mix to each well of the microplate.

Fluorescence was measured at 30°C using a Synergy 4 Multi-Mode Microplate Reader (BioTek Instruments). Kinetic measurements were made every 30 seconds for a total of 60 minutes, while endpoint measurements were made after a 30-minute incubation.

Results

As demonstrated in Figure 2, the Synergy 4 can detect changes in fluorescence as a result of Syk kinase activity. The fluorescence of each reaction increases with reaction time. In addition the fluorescence intensity is dependent on peptide substrate concentration.

Reactions with 3.1- or 12.5- μM peptide substrate increase until a steady state is achieved, with 12.5 increasing to a greater extent, suggesting saturation. The 50- μM substrate concentration samples increased to the greatest extent, but had not reached saturation within 60 minutes.

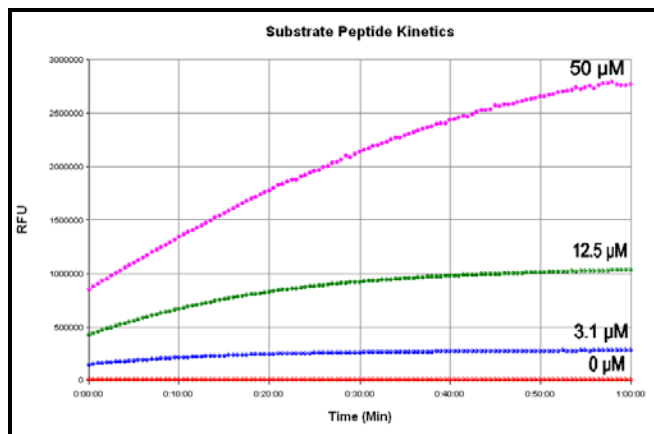


Figure 2. Fluorescent signal generated with different peptide concentrations.

The relationship between the V_{max} rate and the peptide substrate concentration can be described using a 4-parameter logistic fit (Figure 3). As substrate concentration increases the V_{max} of the reaction also increases. These assays can also be measured statically as an end-point determination. When the fluorescence of the reaction is measured at 30 minutes after the initiation of the reaction, the signal is proportional to the substrate concentration (Figure 4). This relationship can also be described using a 4-parameter logistic fit of the data.

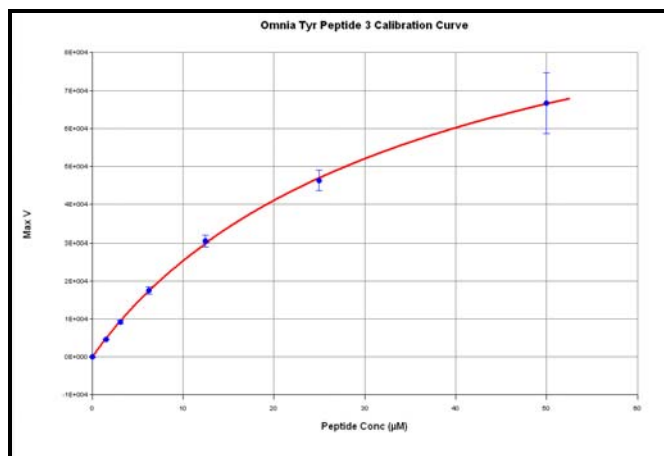


Figure 3. Omnia[®] Tyr Peptide 3 substrate concentration plotted against V_{max} .

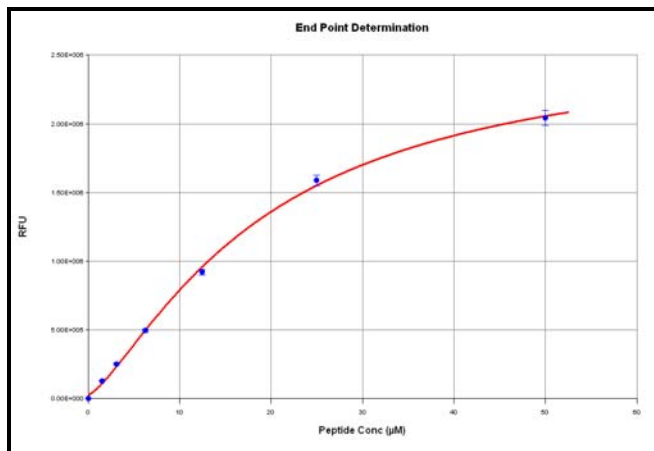


Figure 4. Effect of peptide substrate concentration on fluorescence. Various concentrations of peptide substrate are incubated with a constant amount of Syk protein kinase and the fluorescence measured after a 30-minute incubation.

If the kinase enzyme concentration is varied rather than the substrate concentration, one can observe different rates of fluorescence production that is related to kinase concentration. When 40 ng/well of Syk kinase is present a rapid increase in fluorescence over time is observed, with saturation being reached within 20 minutes. Reactions with 20 ng of kinase protein also increase to saturation, but the process requires over 40 minutes to occur. Likewise, reactions with 10 ng of kinase also show an increase in fluorescence over time, whereas the samples with no kinase demonstrate little to no fluorescence (Figure 5).

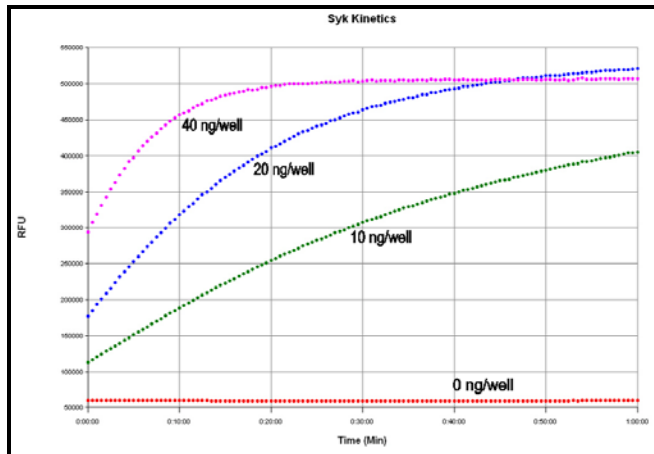


Figure 5. Fluorescence of Omnia[®] reactions with various concentrations of Syk kinase.

Figure 6 demonstrates the ability of the assay to discriminate between 10 μM of substrate and 10 μM of phosphopeptide. The well-to-well variation for either sample is very low with %CV typically less than 5%. When a signal-to-background or a signal-to-noise calculation very high ratios are observed, 11.3 and 79.4 respectively. A Z' value calculation of 0.96 is also seen.

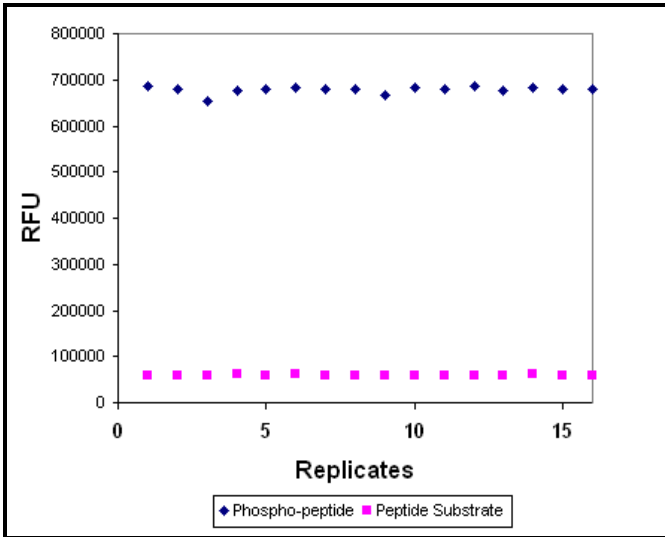


Figure 6. Statistical plot comparing phosphopeptide control to peptide substrate. The fluorescence of 16 samples of both phosphopeptide control and peptide substrate at 10- μ M concentration were measured and plotted.

Statistical Analysis	Result
Signal/Background ratio	11.3
Signal/Noise ratio	79.4
%CV	1.14
Z' value	0.96

Table 1. Statistical calculations based on the comparison between a phosphopeptide control and peptide substrate. The data presented in Figure 6 was used for the determination of these statistical parameters.

Omnia[®] is a registered trademark of Invitrogen.

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