Fluorimetric detection of protein kinase activities and screening of protein kinase inhibitors by monitoring ADP formation

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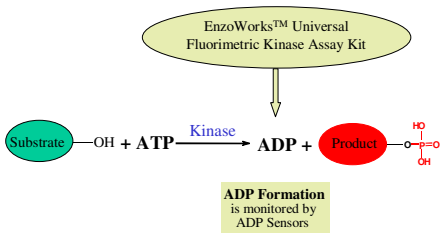
Introduction

Kinases are of interest to researchers involved in drug discovery due to their broad relevance to diseases. Most of existing kinase assays is either based on monitoring of phosphopeptide formation or ATP deletion. For the kinase assays that are based on detection of phosphopeptides one has to spend time and efforts to identify an optimized peptide substrate while the ATP depletion method suffers various interferences due to the use of luciferase that are inhibited or activated by various biological compounds. We have developed a universal fluorimetric protein kinase assay that is based on the monitoring of ADP formation, which is directly proportional to kinase activity and is measured fluorimetricaly with our ADP sensing system. This assay provides a fast, simple, and homogeneous assay for measuring kinase activities and screening kinase inhibitors. For this kinase assay, substrates can be proteins, peptides or sugars, and ATP can be used up to 300 µM.

EnzoWorks™ Kinase Assay Principle



Kinase activity is determined by the monitoring of ADP formation using our fluorescent ADP sensors. ABD Bioquest offers three ABD sensors that can be used at the following wavelengths:
ABD Sensor Red: Ex/Em = 540 nm/590 nm (pH-sensitive)
ADP Sensor UltraRed: Ex/Em = 545 nm/596 nm (pH-insensitive)
ADP Sensor NIR: Ex/Em = 645 nm/670 nm (pH-insensitive)

Material and Methods

1. All Standard dilutions and kinase reactions were performed at room temperature according to the instructions of EnzoWorks™ Universal Fluorimetric Kinase Assay Kit.
2. Standard curves and kinase reactions were performed in 20 µL volumes in Costar 384-solid black well plates for 30 min.
3. ADP was measured using NOVOstar microplate reader (BMG LabTech) at EX 540/Em 590.

Run kinase reaction (20 µL)
↓
Add component A (20 µL)
↓
Add component B (10 µL)
↓
Incubate at room temperature for 15 min-1 hr
↓
Read fluorescence at Ex 540 nm/Em 590 nm

Results

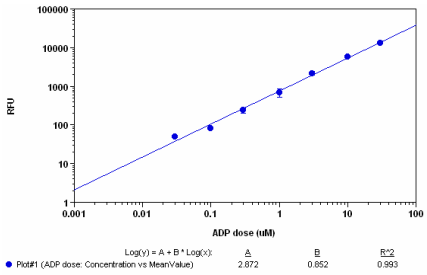


Figure1. ADP dose response was measured with EnzoWorks™ Universal Fluorimetric Kinase Assay Kit in 384-well black plate using NOVOstar microplate reader (BMG LabTech). As low as 0.3 µM ADP can be detected with 15, 30 minutes and 1 hour incubation time (Z' factor =0.65).

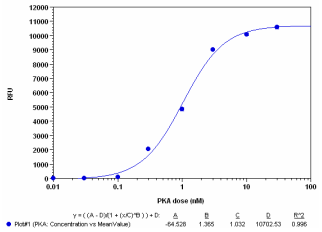


Figure 2. Protein kinase A detection with EnzoWorks™ Universal Fluorimetric Kinase Assay Kit. The kinase was incubated in the presence of ATP and kemptide peptide substrate for 30 minutes, and ADP generation was detected at 30 min incubation time using the EnzoWorks™ Universal Fluorimetric Kinase Assay Kit.

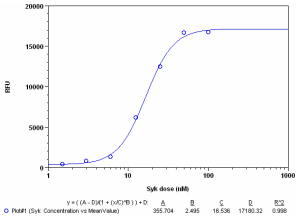


Figure 3. Syk kinase detection with EnzoWorks™ Universal Fluorimetric Kinase Assay Kit. The kinase was incubated in the presence of ATP and Syk peptide substrate for 30 minutes, and ADP generation was detected at 30 min incubation time using the EnzoWorks™ Universal Fluorimetric Kinase Assay Kit.

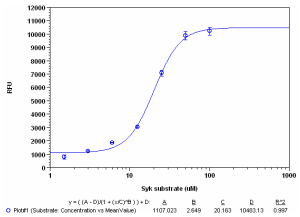


Figure 4. Syk kinase substrate dose response curve on Syk kinase assay reaction. A serial dilutions of Syk substrate was incubated in the presence of 50 uM ATP and 10 nM of Syk kinase for 30 minutes, and ADP generation was detected at 30 min incubation time using the EnzoWorks™ Universal Fluorimetric Kinase Assay Kit.

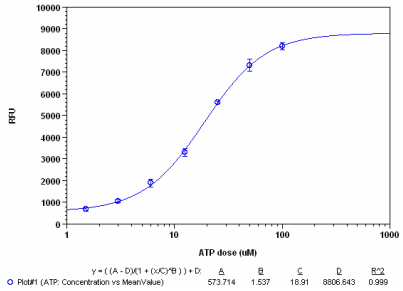


Figure 5. ATP dose response curve on Syk kinase assay reaction. A serial dilutions of ATP was incubated in the presence of 20 nM Syk kinase and 30 uM Syk kinase substrate for 30 minutes, and ADP generation was detected at 30 min incubation time using EnzoWorks™ Universal Fluorimetric Kinase Assay Kit.

Conclusions

1. **EnzoWorks™ Universal Fluorimetric Kinase Assay is a sensitive, fast, simple, and homogeneous assay for measuring a variety of kinase activities.**
2. **As low as 0.3 µM ADP can be detected.**
3. **The assay can be used to measure a variety of kinase activities using the native substrates that can be proteins, peptides or sugars.**
4. **The assay has high tolerance of ATP (used up to 300 µM).**

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