

EnzyChrom™ Kinase Assay Kit (EKIN-400)

A Generic Fluorimetric High-Throughput Kinase Assay

DESCRIPTION

KINASES, also known as phosphotransferases, constitute a large family of enzymes that transfer phosphate groups from the high-energy donor molecule ATP, to their specific substrates. Kinases are known to regulate the majority of cellular processes. The largest group of this family is the protein kinases. So far, 518 different kinases have been identified in humans and up to 30% of human proteins are modified by these kinases. The enormous diversity and their key role in cellular signaling make them ideal targets for drug developments.

BioAssay Systems' EnzyChrom™ Kinase Assay Kit provides a simple and rapid method for assaying kinase activity and high-throughput screening for kinase inhibitors. This homogeneous microplate-based assay involves incubating the kinase with a single working reagent, in which ADP is enzymatically converted to ATP and pyruvate, which is quantified using a fluorimetric (530nm/590nm) assay method.

KEY FEATURES

Safe. Non-radioactive assay.

Sensitive and accurate. As low as 0.01 U/L kinase can be quantified.

Homogeneous and convenient. "Mix-incubate-measure" type assay. The whole assay involves adding a single working reagent and incubation for 10 min at room temperature.

Robust and amenable to HTS: Assay can tolerate up to 300 μM ATP and 10% dimethylsulfoxide (DMSO). Z' factors of > 0.6 are routinely observed in 96/384-well plates. Can be readily automated on HTS liquid handling systems for tens of thousands of assays per day.

APPLICATIONS

Kinase activity assays and HTS for kinase inhibitors.

ADP determination in cells and other biological samples.

KIT CONTENTS

Reagent A: 10 mL **Reagent B:** 10 mL
Assay Buffer: 25 mL **Standard:** 100 μL 3 mM ADP

Storage conditions: The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

This kit is sufficient for 400 assays in 384-well plate (200 assays in 96-well plate). Use *black flat-bottom* plates. Prior to assay, bring all reagents to room temperature. Assays in duplicate wells are recommended.

Interference: thiols (β-mercaptoethanol, dithioerythritol etc) at > 10 μM interfere with this assay and should be avoided.

Kinase Activity Assay in 384-well Plate

- Kinase Reaction.** Users should provide their own enzyme, ultra-pure ATP (e.g. Sigma # A7699) and substrate. Set up 20 μL reaction mixture containing the kinase, ATP and substrate in the provided Assay Buffer (pH 7.0) or any suitable kinase buffer. Set up a Blank Control that contains ATP and substrate but no enzyme. Incubate at desired temperature for desired period of time e.g. 30 min.
- Standards.** Prepare 900 μL 10 μM ADP Premix by mixing 3 μL 3 mM Standard and 897 μL distilled water. Dilute standard as follows. Transfer 20 μL standards into separate wells of the plate.

No	Premix + H ₂ O	Vol (μL)	ADP (μM)
1	100 μL + 0 μL	100	10
2	60 μL + 40 μL	100	6
3	30 μL + 70 μL	100	3
4	0 μL + 100 μL	100	0

- ADP Detection.** Prepare enough Working Reagent for each well by mixing 25 μL Reagent A and 25 μL Reagent B. Add 40 μL Working Reagent to each assay well. Tap plate to mix. Incubate at room temperature for 10 min.

- Read fluorescence intensity** at λ_{exc} = 530nm and λ_{em} = 590nm. Calculate kinase activity,

$$\text{Kinase Activity} = \frac{\Delta F_{\text{SAMPLE}}}{\text{Slope} \cdot t} \times \frac{20 \mu\text{L}}{\text{Vol} (\mu\text{L})} \text{ (U/L)}$$

where ΔF_{SAMPLE} = (fluorescence intensity of sample well – fluorescence of the blank well), slope is the slope of the ADP standard curve. t is the kinase reaction time (e.g. 30 min). Vol is the volume (μL) of kinase added to the 20 μL reaction.

Note: typical kinase assays use 100 to 200 μM ATP. If ΔF_{SAMPLE} > (F_{10μM ADP} – F_{H₂O}), dilute enzyme in assay buffer. Repeat the assay, multiply the results by the dilution factor n. This will ensure that the initial rate is measured at < 10% ATP conversion.

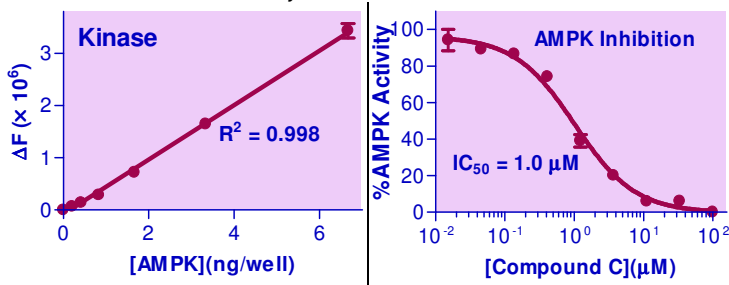
High-throughput Screen for Kinase Inhibitors

For inhibitor screens, test compounds are usually pre-incubated with kinase for 10 to 30 min, prior to adding ATP/substrate to initiate kinase reaction. After a 30-min kinase reaction, the produced ADP is quantified using the fluorimetric assay. The fluorescence intensity will be decreased in the presence of an inhibitor.

- Controls and compounds.** Use a known kinase inhibitor (e.g. staurosporine) as a positive control. Alternatively, "no enzyme" wells can serve as a positive control. Use the same volume of the compound solvent (e.g. DMSO) as an inhibitor negative control.

Example: transfer 5 μL test compound, control inhibitor and negative control (e.g. DMSO) to appropriate wells, add 10 μL enzyme solution to all assay wells. Apply an "in-well" mixing step. Incubate for 10 to 30 min.

- Kinase Reaction.** Add 5 μL mixture containing ATP and the kinase substrate to each assay well. Mix well. Incubate for desired period of time (e.g. 30 min).
- ADP Detection.** Prepare enough Working Reagent by mixing 25 μL Reagent A and 25 μL Reagent B. Add 40 μL Working Reagent to each well and mix well. Incubate for 10 min.
- Read fluorescence intensity** at λ_{exc} = 530nm and λ_{em} = 590nm.



Left: Enzyme titration with AMPK. Right: Inhibition curve with 2 ng AMPK and 50 μM ATP

PUBLICATIONS

- Liu, K et al. (2020). Different p53 genotypes regulating different phosphorylation sites and subcellular location of CDC25C associated with the formation of polyploid giant cancer cells. *Journal of Experimental & Clinical Cancer Research*: CR, 39(1), 83.
- Hirooka, K. et al. (2016). Regulation of the rhaEWRBMA operon involved in l-rhamnose catabolism through two transcriptional factors, RhaR and CcpA, in *Bacillus subtilis*. *Journal of bacteriology*, 198(5), 830-845.

