**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 achieved.
- Sensitive and accurate. 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 adapted 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**

1. **Kinase Reaction**: Users should provide their own enzyme, ultra-pure ATP (e.g. Sigma # A7909) 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).

2. **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.

<table>
<thead>
<tr>
<th>No</th>
<th>Premix + H2O</th>
<th>Vol (µL)</th>
<th>ADP (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µL + 0 µL</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>60 µL + 40 µL</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>30 µL + 70 µL</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>0 µL + 100 µL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

3. **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.

4. **Read fluorescence intensity** at λexc = 530nm and λem = 590nm. Calculate kinase activity,

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

   where \(\Delta F_{\text{SAMPLE}}\) = (fluorescence intensity of sample well – fluorescence intensity 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.

**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.

1. **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.

2. **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).


4. **Read fluorescence intensity** at λexc = 530nm and λem = 590nm.

**LITERATURE**
