Creatine Kinase Activity Assay Kit

Catalog Number MAK116
Storage Temperature –20 °C

Product Description
Creatine Kinase (CK), also known as phosphocreatine kinase, is an enzyme that catalyzes the transfer of one phosphate group from ATP to creatine generating phosphocreatine, an important energy reservoir in muscle and brain tissue. CK is a dimeric protein made up of B (brain) and M (muscle) subunits. Three isoenzymes, CK-MM, CK-MB, and CK-BB, have been observed. CK levels are elevated in various pathological conditions including myocardial infarction, rhabdomyolysis, muscular dystrophy, and renal failure.

The Creatine Kinase Activity Assay kit provides a simple and direct procedure for measuring CK levels in a variety of samples such as blood, serum, and plasma. In this assay, Creatine Kinase activity is determined by a coupled enzyme reaction resulting in the production of NADPH, measured at 340 nm, proportionate to the CK activity present in the sample. In this reaction, phosphocreatine and ADP are converted to creatine and ATP. The generated ATP is used by hexokinase to phosphorylate glucose resulting in glucose-6-phosphate, which is oxidized by NADP in the presence of glucose-6-phosphate dehydrogenase to produce NADPH and 6-phospho-D-gluconate. One unit of CK is the amount of enzyme that will transfer 1.0 µmole of phosphate from phosphocreatine to ADP per minute at pH 6.0. This kit has a linear range of 30–1,800 units/L CK activity.

Components
The kit is sufficient for 100 assays in 96 well plates.

- Assay Buffer
  Catalog Number MAK116A
  12 mL

- Enzyme Mix
  Catalog Number MAK116B
  120 µL

- Substrate Solution
  Catalog Number MAK116C
  1 mL

- Calibrator
  Catalog Number MAK116D
  150 µL

Reagents and Equipment Required but Not Provided.
- Ultraviolet Spectrophotometric multiwell plate reader
- Clear 96 well flat-bottom plate suitable for use in UV absorbance assays (Catalog No. CLS3635 or equivalent).

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
This kit is shipped on dry ice. Storage at –20 °C, protected from light, is recommended.

Procedure
Sample Preparation
Blood samples should be not be hemolyzed and assayed within 4 hours of collection if stored at room temperature and 12 hours if samples are stored at 2–8 °C. Alternatively, samples can be stored at –80 °C.

Tissue samples should be rinsed in phosphate-buffered saline, pH 7.4, to remove blood. Homogenize tissue (50 mg) in 200 µL of 50 mM postassium phosphate, pH 7.5, buffer. Centrifuge at 10,000 × g for 15 minutes at 2–8 °C. Use cleared supernatant for assay.

Collect cells by centrifugation at 2,000 × g for 5 minutes at 2–8 °C. For adherent cells, do not harvest using proteolytic enzymes, instead use a cell scraper.

Homogenize or sonicate cells in an appropriate volume of cold 50 mM potassium phosphate, pH 7.5, buffer. Centrifuge at 10,000 × g for 15 minutes at 2–8 °C. Remove supernatant for assay.
All samples can be stored at –80 °C for up to one month.

**Assay Reaction**

1. This reaction can be carried out at either room temperature or 37 °C. Bring all components to room temperature or 37 °C before use. Prepare enough of the Reconstituted Reagent for each sample to be tested according to the scheme in Table 1. Each sample requires 100 µL of Reconstituted Reagent.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
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</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>100 µL</td>
</tr>
<tr>
<td>Substrate Solution</td>
<td>10 µL</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>1 µL</td>
</tr>
</tbody>
</table>

2. Transfer 110 µL of water into one well (Blank) and 100 µL of water plus 10 µL of the Calibrator into a separate well of a 96 well plate.

3. Transfer 10 µL of samples into separate wells. Add 100 µL of the Reconstituted Reagent to each sample well and tap plate to mix.

4. Incubate the samples at either room temperature or 37 °C. After 20 minutes, take the initial absorbance measurement at 340 nm (A\textsubscript{340}\text{initial}).

   **Note:** CK is fully activated within 20 minutes by the glutathione present in the Substrate Solution.

5. Continue to incubate the plate at either room temperature or 37 °C for 20 additional minutes. Measure the (A\textsubscript{340}\text{final}).

   **Note:** If the CK activity is expected to be higher than 300 units/L, measure the A\textsubscript{340} at 5 minutes past the initial measurement.

**Calculations**

CK Activity (units/L) = \( \frac{(A_{340})_{\text{final}} - (A_{340})_{\text{initial}} \times 150}{(A_{340})_{\text{calibrator}} - (A_{340})_{\text{blank}}} \)

where:

150 = equivalent activity (units/L) of the Calibrator when assay is read at 20 minutes and 40 minutes (20 additional minutes past initial reading).

**Note:** If the CK activity is expected to be higher than 300 units/L, read A\textsubscript{340} at 20 minutes and again at 25 minutes. To calculate the CK activity, replace \([A_{340}]_{40 \text{ min}} - (A_{340})_{20 \text{ min}}\) with \([A_{340}]_{25 \text{ min}} - (A_{340})_{20 \text{ min}}\) and replace the factor 150 with 600 in the above equation.

Linear range: 30–1,800 units/L of CK activity

One unit of CK is the amount of enzyme that will transfer 1.0 µmole of phosphate from phosphocreatine to ADP per minute at pH 6.0.

**Troubleshooting Guide**

<table>
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<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
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<tbody>
<tr>
<td>Assay not working</td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
</tr>
<tr>
<td></td>
<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
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<tr>
<td></td>
<td>Type of 96 well plate used</td>
<td>For UV assays, use clear plates that are UV transparent or quartz plates.</td>
</tr>
<tr>
<td>Samples with erratic readings</td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
</tr>
<tr>
<td></td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
</tr>
</tbody>
</table>

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