

**Creatine Kinase Fluorometric
Assay Kit**

Item No. 700630

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GENERAL INFORMATION

Materials Supplied

The kit will arrive as two packages at different shipping temperatures. The Formaldehyde Standard has to be stored at $\geq 4^{\circ}\text{C}$ or it may polymerize. For best results, remove components and store as stated below.

| Item Number | Item | Quantity/Size | Storage |
|-------------|---|--------------------------|------------------|
| 700621 | Potassium Phosphate Buffer (500 mM; pH 7.0) | 1 vial/5 ml | -20°C |
| 700631 | Creatine Kinase Positive Control | 2 vials | -20°C |
| 700633 | Creatine Kinase Enzyme Mixture | 2 vials | -20°C |
| 700634 | Creatine Phosphate | 1 vial/10 mg | -20°C |
| 700635 | Creatine Kinase ADP | 2 vials | -20°C |
| 700381 | Formaldehyde Detector | 1 vial/300 mg | Room temperature |
| 700382 | Formaldehyde Ammonium Acetate | 1 vial/10 ml | Room temperature |
| 700384 | Formaldehyde Standard | 1 vial/100 μl | Room temperature |
| 700001 | DMSO Assay Reagent | 1 vial/3 ml | Room temperature |
| 400017 | 96-Well Solid Plate (black) | 1 plate | Room temperature |
| 400012 | 96-Well Cover Sheet | 1 cover | Room temperature |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

Formaldehyde is carcinogenic. It is toxic if inhaled, ingested, or if in contact with skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. Keep away from combustible materials.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence at an excitation wavelength between 365-375 nm and an emission wavelength between 465-475 nm
2. Adjustable pipettes and a repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Creatine kinase (CK), also called creatine phosphokinase (CPK), catalyzes the reversible phosphorylation of creatine by ATP to form phosphocreatine and ADP.^{1,2} Phosphocreatine is the major storage form of high energy phosphate in muscle. In cells, cytosolic CK enzymes consist of two subunits, which can be either B (brain-type) or M (muscle-type). There are three CK isoenzymes: CK-BB (CPK-1) is mainly produced by the brain and lungs; CK-MB (CPK-2) is primarily produced by heart muscle; and most of CK-MM (CPK-3) is produced by skeletal muscle.^{1,2} In addition to those three cytosolic CK isoforms, there are two mitochondrial creatine kinase isoenzymes, the ubiquitous and sarcomeric form.¹

Under normal conditions, there is very little CK circulating in the blood of the average, healthy human being. Clinically, CK (in blood) is assayed as a marker of myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, and acute renal failure.³⁻⁵ Some medications which are commonly used to decrease serum cholesterol levels (statins), may be associated with CK elevation in about 1% of the patients.^{6,7} There is an inverse relationship in the serum levels of T3 and CK in thyroid disease.^{8,9} In hypothyroid patients with a decrease in serum T3, there is a significant increase in CK. Therefore, the estimation of serum CK activity is considered valuable in screening for hypothyroid patients. Lowered CK activity can be an indication of alcoholic liver disease and rheumatoid arthritis.^{10,11}

About This Assay

Cayman's Creatine Kinase Assay provides a convenient method for detecting total CK activity in plasma and serum. CK catalyzes the reversible reaction between creatine phosphate and ADP to form creatine and ATP. Through a series of enzyme-coupled reactions, formaldehyde is generated. The cyclization of formaldehyde and acetoacetanilide in the presence of ammonia results in a fluorescent product which is analyzed using an excitation wavelength of 365-375 nm and an emission wavelength of 465-475 nm (see Figure 1 on page 6).¹²

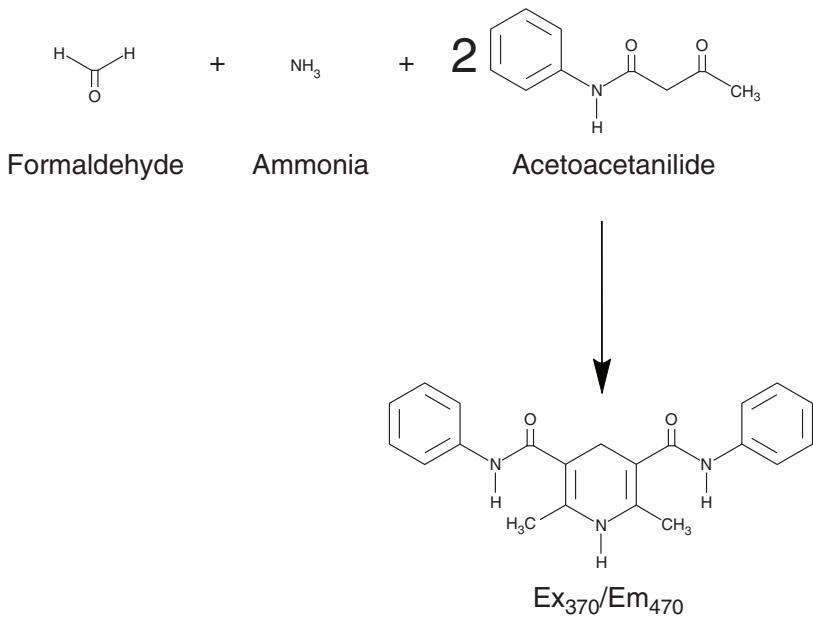


Figure 1. Formaldehyde detection

PRE-ASSAY PREPARATION

Reagent Preparation

1. Potassium Phosphate Buffer (500 mM; pH 7.0) - (Item No. 700621)

The vial contains 5 ml of 500 mM potassium phosphate, pH 7.0. Dilute the contents of the vial with 45 ml of HPLC-grade water. This final Buffer (50 mM potassium phosphate, pH 7.0) is used in the assay and for diluting reagents. When stored at 4°C, this diluted buffer is stable for three months.

2. Creatine Kinase Positive Control - (Item No. 700631)

Each vial contains a lyophilized powder of creatine kinase (creatin phosphokinase). Reconstitute the contents of the vial with 200 µl of diluted Buffer and store on ice. The reconstituted enzyme is stable for four hours at 4°C.

3. Creatine Kinase ADP - (Item No. 700635)

Each vial contains a lyophilized powder of adenosine 5'-diphosphate (ADP). Reconstitute the contents of the vial with 600 µl of diluted Buffer. One vial of ADP is sufficient to assay 60 wells. Reconstitute the additional vial if assaying the entire plate. The reconstituted ADP is stable for one day at -20°C.

4. Creatine Kinase Enzyme Mixture - (Item No. 700633)

Each vial contains a lyophilized powder of enzymes. Reconstitute the contents of the vial with 600 µl of diluted Buffer and store on ice. One vial of Enzyme Mixture is sufficient to assay 60 wells. Reconstitute the additional vial if assaying the entire plate. The reconstituted reagent is stable for one day at -20°C.

5. Creatine Phosphate - (Item No. 700634)

The vial contains 10 mg of creatine phosphate. In a separate vial, weigh out 4 mg of Creatine Phosphate. Dissolve the substrate with 1.2 ml of diluted Buffer to yield a concentration of 10 mM. This is sufficient substrate to assay 60 wells. Prepare additional Creatine Phosphate as needed. The Creatine Phosphate solution is stable for eight hours at room temperature.

6. Formaldehyde Detector - (Item No. 700381)

The vial contains 300 mg of acetoacetanilide. Prior to assaying, weigh 130 mg into another vial, add 1.2 ml of DMSO, and vortex until dissolved. This is sufficient reagent to assay 60 wells. Prepare additional reagent if assaying the entire plate. Any unused reconstituted Detector can be stored at -20°C for one month.

7. Formaldehyde Ammonium Acetate - (Item No. 700382)

The vial contains 10 ml of ammonium acetate. The reagent is ready to use in the assay.

8. Formaldehyde Standard - (Item No. 700384)

The vial contains 100 µl of 3 M formaldehyde. See page 12 for diluting the Standard prior to preparing the standard curve. *Do not freeze!*

9. DMSO Assay Reagent - (Item No. 700001)

The vial contains 3 ml of dimethylsulfoxide (DMSO). It is ready to use to prepare the Formaldehyde Detector.

Sample Preparation

Plasma

Typically, normal human plasma has a creatine kinase activity of 10-200 U/L.

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 25°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer.
3. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
4. Dilute plasma 1:5-1:20 with diluted Buffer before assaying.

Serum

Typically, normal human serum has a creatine kinase activity of 10-200 U/L.

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 25°C. Pipette off the top yellow serum layer without disturbing the white buffy layer.
4. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
5. Dilute serum 1:5-1:20 with diluted Buffer before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a formaldehyde standard curve in duplicate has to be assayed with the samples. We suggest that each sample be assayed at least in duplicate. It is recommended to assay two wells of the Creatine Kinase Positive Control. A typical layout of standards, positive control, and samples to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 23).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | A | A | S1 | S1 | S9 | S9 | S17 | S17 | S25 | S25 | S33 | S33 |
| B | B | B | S2 | S2 | S10 | S10 | S18 | S18 | S26 | S26 | S34 | S34 |
| C | C | C | S3 | S3 | S11 | S11 | S19 | S19 | S27 | S27 | S35 | S35 |
| D | D | D | S4 | S4 | S12 | S12 | S20 | S20 | S28 | S28 | S36 | S36 |
| E | E | E | S5 | S5 | S13 | S13 | S21 | S21 | S29 | S29 | S37 | S37 |
| F | F | F | S6 | S6 | S14 | S14 | S22 | S22 | S30 | S30 | S38 | S38 |
| G | G | G | S7 | S7 | S15 | S15 | S23 | S23 | S31 | S31 | S39 | S39 |
| H | + | + | S8 | S8 | S16 | S16 | S24 | S24 | S32 | S32 | S40 | S40 |

A-G = Standards

+ = Positive Control Wells

S1-S40 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 μ l in all the wells.
- All reagents except the enzymes must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples at least in duplicate (triplicate preferred).
- The assay has two incubation temperatures: 37°C and room temperature.
- Monitor the fluorescence with an excitation wavelength of 365-375 nm and an emission wavelength of 465-475 nm.

Standard Preparation

Dilute 10 μ l of the 3 M formaldehyde standard with 990 μ l of HPLC-grade water to yield 30 mM formaldehyde. Dilute 25 μ l of the 30 mM Formaldehyde with 1.475 ml of HPLC-grade water to yield the 500 μ M Formaldehyde Stock Solution. Take eight clean glass test tubes or polystyrene tubes and mark them A-G. Add the amount of 500 μ M Formaldehyde Stock and HPLC-grade water to each tube as described in Table 1. The diluted Standards are stable for two hours at room temperature.

| Tube | 500 μ M Formaldehyde Stock (μ l) | HPLC-grade water (μ l) | Final Concentration (μ M) |
|------|---|-----------------------------|--------------------------------|
| A | 0 | 1,000 | 0 |
| B | 20 | 980 | 10 |
| C | 50 | 950 | 25 |
| D | 100 | 900 | 50 |
| E | 150 | 850 | 75 |
| F | 200 | 800 | 100 |
| G | 300 | 700 | 150 |

Table 1. Preparation of Formaldehyde Standards

Performing the Assay

1. **Standard Wells** - Add 50 μ l of diluted Buffer, 10 μ l of standard (tubes A-G), per well in the designated wells on the plate (see **Sample Plate Format**, Figure 2, page 10).
2. **Positive Control Wells** - Add 50 μ l of diluted Buffer and 10 μ l of CK Positive Control to at least two wells.
3. **Sample Wells** - Add 50 μ l of diluted Buffer and 10 μ l of sample to at least two wells
4. Add 10 μ l of ADP to all wells being used.
5. Add 10 μ l of Enzyme Mixture to all wells being used.
6. Initiate the reactions by adding 20 μ l of Creatine Phosphate to all wells being used.
7. Cover the plate with the plate cover and incubate for 15 minutes at 37°C.
8. Remove the plate cover and add 80 μ l of Ammonium Acetate and 20 μ l of Formaldehyde Detector to every well being used.
9. Cover the plate with the plate cover and incubate for 15 minutes at room temperature.
10. Remove the plate cover and read the plate at an excitation wavelength of 365-375 nm and an emission wavelength of 465-475 nm.

| Reagent | Standard Wells (µl) | Sample Wells (µl) | Positive Control Wells (µl) |
|---|---------------------|-------------------|-----------------------------|
| Diluted Buffer | 50 | 50 | 50 |
| Standards | 10 | - | - |
| CK Positive Control | - | - | 10 |
| Sample | - | 10 | - |
| ADP | 10 | 10 | 10 |
| Enzyme Mixture | 10 | 10 | 10 |
| Creatine Phosphate | 20 | 20 | 20 |
| Incubate for 15 min at 37°C | | | |
| Ammonium Acetate | 80 | 80 | 80 |
| Formaldehyde Detector | 20 | 20 | 20 |
| Incubate for 15 min at room temperature | | | |

Table 2. Pipetting summary

ANALYSIS

Calculations

1. Determine the average fluorescence of each standard, positive control, and sample.
2. Subtract the fluorescence value of standard A (0 µM) from itself and all other standards. This is the corrected fluorescence (RFU).
3. Plot the corrected fluorescence values (from step 2 above) of each standard as a function of the final formaldehyde concentration (µM) from Table 1. See Figure 3, on page 16, for a typical standard curve.
4. Calculate the CK activity for each sample and the positive control using the equation below. One unit is defined as the amount of enzyme that will cause the formation of 1 µmol of formaldehyde per minute at 37°C.

$$\text{Creatine Kinase (U/L)} = \left[\frac{\text{RFU}/15 \text{ min} - (\text{y-intercept})}{\text{Slope from formaldehyde curve (RFU/µM)}} \right] \times \text{Sample dilution}$$

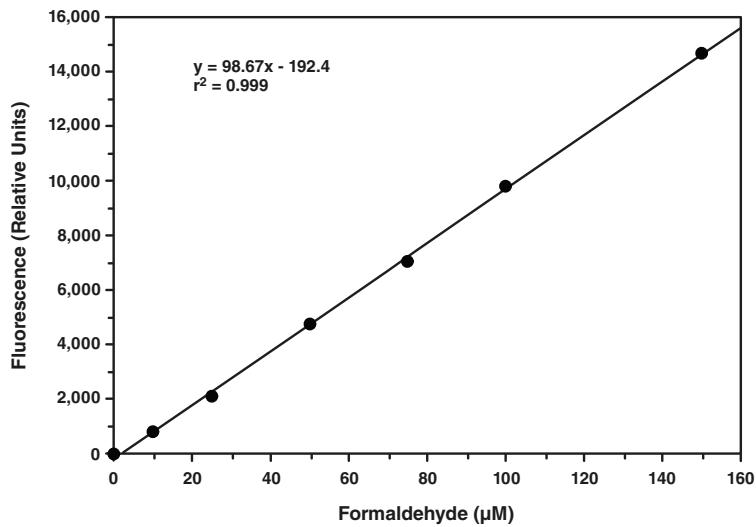


Figure 3. Formaldehyde standard curve

Performance Characteristics

Sensitivity:

The limit of detection for the assay is 2 U/L (± 0.5 U/L) creatine kinase.

Precision:

When a series of 16 human plasma measurements were performed on the same day, the intra-assay coefficient of variation was 3.5%. When a series of 16 human plasma measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 3.1%, respectively.

Spiking Data:

Plasma was spiked with various concentrations of creatine kinase. Creatine kinase activity was then determined for each creatine kinase concentration. The data in Figure 4, on page 18, represents the amount of creatine kinase added to plasma *versus* the calculated amount of creatine kinase.

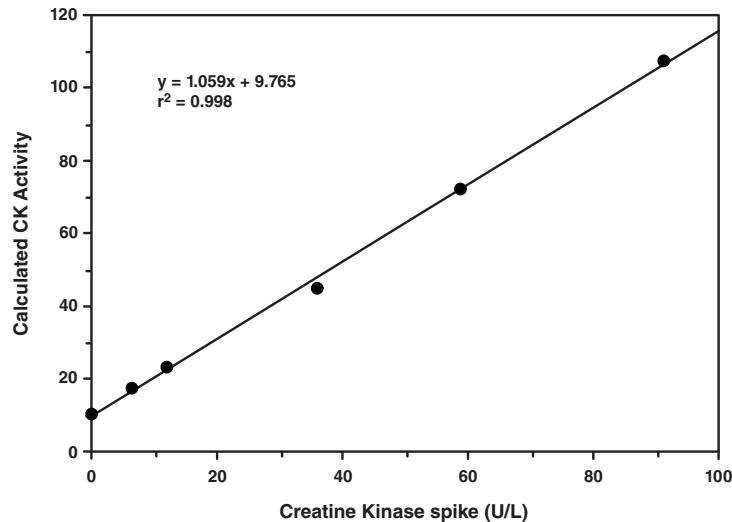


Figure 4. Spiking experiment

RESOURCES

Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|--|
| Erratic values; dispersion of duplicates/triplicates | A. Poor pipetting/technique B. Bubble in the well(s) | A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles |
| No fluorescence was detected above background in the sample wells | Sample was too dilute | Re-assay the sample using a lower dilution |
| The fluorescence of the sample wells were higher than the last standard | Sample was too concentrated | Re-assay the sample using a higher dilution |
| The fluorometer exhibited "MAX" values for the wells | The GAIN setting is too high | Reduce the GAIN and re-read |

References

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Related Products

- Cholesterol Assay Kit - Item No. 10007640
- Cholesterol Cell-Based Detection Assay Kit - Item No. 10009779
- ChREBP Transcription Factor Assay Kit - Item No. 10006909
- Coenzyme A Assay Kit - Item No. 700440
- Creatinine (serum) Assay Kit - Item No. 700460
- Creatinine (urinary) Assay Kit - Item No. 500701
- Free Fatty Acid Assay Kit - Item No. 700310
- Glucose Colorimetric Assay Kit - Item No. 10009582
- Glucose Fluorometric Assay Kit - Item 700710
- Glucose-6-Phosphate Dehydrogenase Activity Assay Kit - Item No. 700300
- Glycerol Colorimetric Assay Kit - Item No. 10010755
- Glycerol Fluorometric Assay Kit - Item No. 700720
- Glycogen Assay Kit - Item No. 700480
- β -Hydroxybutyrate (Ketone Body) Assay Kit - Item No. 700190
- D-Lactate Assay Kit - Item No. 700520
- L-Lactate Assay Kit - Item No. 700510
- oxLDL- β 2GPI (human) ELISA Kit - Item No. 10008793
- Lipid Droplets Fluorescence Assay Kit - Item No. 500001
- Pyruvate Assay Kit - Item No. 700470
- Triglyceride Colorimetric Assay Kit - Item No. 10010303
- Triglyceride Fluorometric Assay Kit - Item No. 700730

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

NOTES

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