

Certain drugs and medications may affect the activity of CK, see Young et al.¹⁰

MATERIALS REQUIRED BUT NOT PROVIDED

Sample and reagent pipettes, test vials or cuvettes, timer, test tube rack, 37°C heating bath, control serum, 0.1 N Hydrochloric Acid, spectrophotometer.

PROCEDURE (MANUAL)

Reconstitute CK reagent according to instructions

1. Transfer 0.5 ml of CK Reagent to test tubes labeled: UNKNOWN(S), CONTROL(S), CALIBRATOR, and REAGENT BLANK.
2. Prewarm tubes at 37°C for 3 - 5 minutes.
3. At timed intervals add 0.010 ml (10 ul) of sample to its respective tube, mix and return to 37°C heating bath for exactly 10 minutes.
4. Following the same timed intervals, add 0.10 ml (100 ul) of CK COLOR REAGENT to all tubes, mix and incubate for five (5) minutes at 37°C.
5. Stop the reaction by adding 2.5 ml of 0.1 N Hydrochloric Acid to all tubes and mix.
6. Zero the spectrophotometer at 500 nm using the REAGENT BLANK. Read and record the absorbance for each vial. (Wavelength range:500-520).

* USE TC - MUTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

PROCEDURE NOTES

1. In the case of very icteric or lipemic serum, a serum blank should be measured. This may be performed as follows:
 - a. Add 0.010 ml (10 ul) of the sample to 3.1 ml of deionized water.
 - b. Zero the spectrophotometer at 500 ± 5 nm with deionized water.
 - c. Read and record absorbance of "serum blank".
 - d. Subtract this "serum blank" absorbance from the sample absorbance measured in step #6 above. Use this corrected absorbance value to calculate CK activity.
2. Traumatic muscle injury (i.e intramuscular injections) as well as vigorous physical exercise, labor, and delivery of pregnancy will elevate the CK value.
3. CK is a light sensitive enzyme and excessive light exposure reportedly will cause CK values to decrease in the serum sample.

PROCEDURE LIMITATIONS

1. Some inhibitors of CK activity¹¹
 - a. Excessive Mg⁺⁺ Cl⁻ SO₄⁻
 - b. Most heavy metals, i.e. Zn⁺⁺, Cu⁺⁺, Mn⁺⁺
 - c. Iodoacetate and other sulfhydryl binding agents
 - d. Excess ADP, citrate, fluoride, L-thyroxine
 - e. Excess uric acid
2. This procedure measures total CK activity irrespective of its tissue or organ of origin.
3. Lower than expected CK values have been reported in samples having high alkaline phosphatase activity.

CALCULATIONS

Use the absorbance readings of the CALIBRATOR and UNKNOWN(S) to calculate CK values as follows:
(where A = absorbance)

$$\frac{A(\text{UNKNOWN})}{A(\text{CALIBRATOR})} \times \text{CK value of CALIBRATOR (IU/L)}$$

$$= \text{CK in unknown (IU/L)}$$

EXAMPLE OF CALCULATION

Assume that the CALIBRATOR had a CK value of 200 IU/L and that it gave an absorbance of 0.15 while the UNKNOWN gave an

absorbance of 0.21. The CK value of the UNKNOWN may then be calculated as follows:

$$\frac{0.21}{0.15} \times 200 \text{ IU/L} = 280 \text{ IU/L}$$

QUALITY CONTROL

Use control sera with known normal and abnormal values to monitor the integrity of the reaction. Values should be those acceptable for this method and temperature.

EXPECTED VALUES¹²

25 - 192 IU/L (37°C)

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

Linearity: 1000 IU/L

Sensitivity: Based on an instrument resolution of A = 0.001, this procedure has a sensitivity of 1.5 IU/L.

Comparison: Studies done between this procedure and a UV procedure yield a correlation coefficient of 0.99 with a regression equation of Y = 0.87x + 12.99.

Precision:

Mean (mg/dl)	Within Run	
	S.D.	C.V.%
154	8.6	5.6
405	22.5	5.5

Mean (mg/dl)	Run to Run	
	S.D.	C.V.(%)
144	8.6	5.9
413	19.1	4.6

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