MEDICA

IVD CE

REF 10222-4 4 x 23 mL / 6 mL

CREATINE KINASE (CK)

Wedges each contain usable volumes of 23 mL of R1 reagent and 6 mL of R2 reagent.

INTENDED USE

The EasyRA CK reagent is intended for the quantitative determination of the enzyme creatine kinase (CK) in human serum or plasma (with lithium heparin as anticoagulant), using the MEDICA "EasyRA® Clinical Chemistry Analyzer." Measurements of CK are used in the diagnosis and treatment of myocardial infarction and muscle diseases such as progressive Duchenne-type muscular dystrophy. For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Creatine kinase is an enzyme whose physiological function is to catalyze the transfer of a phosphate group from adenosine triphosphate (ATP) to creatine. The majority of the creatine kinase in the body is contained in muscle cells. Serum CK activity has proven useful to evaluate cardiac and skeletal muscle diseases. Higher than normal amounts of creatine kinase in serum can indicate muscle damage to heart or other tissue from either chronic disease or acute muscle injury.[1] Intramuscular injections and strenuous exercise can elevate serum CK. Interpretation of the results must be considered in the context of the clinical status of the patient. CK catalyzes the reversible phosphorylation of creatine by ATP. At neutral pH, the reverse reaction occurs with the formation of ATP from creatine phosphate. The kinetic measurement method being used is Szasz's[2] modification of the procedure used by Rosalki,[3] which employs a sequence of coupled enzyme reactions using creatine phosphate as the substrate.

PRINCIPLE OF THE PROCEDURE

CK catalyses the reversible transfer of a phosphate group from the creatine phosphate to ADP forming ATP.

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow[\text{kinase}]{\text{Creatine}} \text{Creatine} + \text{ATP}$$

By the use of two coupled reactions employing hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH), the formation of ATP can be measured. The rate of reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH can be measured spectrophotometrically by the increase in absorbance at 340 nm.

$$\text{ATP} + \text{D-Glucose} \xrightarrow{\text{hexokinase}} \text{Glucose-6-P} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP} \xrightarrow{\text{G6PDH}} \text{D-Gluconate-6-phosphate} + \text{NADPH} + \text{H}^+$$

The rate of the formation of NADPH is directly proportional to the CK activity in the sample.

REAGENTS

CK Buffer Reagent (R1):

Imidazole Buffer (pH 6.7)	100 mmol/L
D-Glucose	20 mmol/L
N-Acetyl-L-Cysteine	20 mmol/L
Magnesium Acetate	10.0 mmol/L
NADP	2.0 mmol/L
EDTA	2.0 mmol/L
Hexokinase (Baker's yeast)	2500 U/L

CK Substrate Reagent (R2):

Imidazole Buffer (pH 6.7)	100 mmol/L
Creatine phosphate	30 mmol/L
ADP	2.0 mmol/L
AMP	5.0 mmol/L
Diadenosine pentaphosphate	10.0 µmol/L
Glucose-6-PDH (Baker's yeast)	1500 U/L
EDTA	2.0 mmol/L

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C . The reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE/STABILITY

Clear unhemolyzed serum or plasma should be used. Centrifuge and remove the serum as soon as possible after collection. Serum CK is stable for 3 days at 2 – 8°C. Lithium heparin coated tubes may be used for plasma collection.

PROCEDURE

Materials Provided

Medica CK Reagent Wedge, REF 10222

Additional materials required

Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use

The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (60 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the EasyRA Analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration

Not applicable.

Quality Control

It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results

After completion of the assay, the EasyRA Analyzer calculates the CK concentration from change in absorbance per minute, sample volume, total reaction volume, pathlength (cm) of 0.6 and molar absorptivity of 6.22.

$$\text{CK (U/L)} = (\Delta A/\text{Min}) \times \frac{(\text{Total Volume}(\mu l) \times 1000)}{(\text{Molar absorptivity} \times \text{Pathlength (cm)} \times \text{Sample Volume}(\mu l))}$$

A unit per liter (U/L) of CK activity is the amount of enzyme, which oxidizes one µmol/L of NADP per minute.

Expected Values[4]

The reference range for CK in serum and plasma is as follows:

	37°C
Adult Male:	24 – 195 U/L
Adult Female:	24 – 170 U/L

These values are guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g., if sample is above assay range)
Avoid using hemolyzed serum or plasma samples.

If the Absorbance Change per Minute (ΔA/Min) is greater than 0.20, which corresponds approximately to 1200 U/L, results will be flagged with "SD" (substrate depletion) by the analyzer. Absorbance changes per minute above this are above the linear range of the test. If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the CK test to 2400 U/L.

PERFORMANCE CHARACTERISTICS[5]

Reportable Range
The reportable range is 7 to 1200 U/L. Extended range is 7 to 2400 U/L when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for CK (y) on the EasyRA Analyzer to the performance of a similar CK reagent (x) on the Roche COBAS MIRA* Analyzer. The data shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate values obtained on the COBAS MIRA Analyzer.

Number of samples	54	Range of Samples	7 to 1182 U/L
Slope	1.0185	y Intercept	5.5223
Correlation Coefficient	0.9912	Regression Equation:	Y = 1.0185*X + 5.5223

The following table lists the data obtained in a comparison of matched serum (x) and plasma (y) samples using the Medica reagent for CK on the EasyRA analyzer. The data below represents a single plasma determination vs. the average of two replicate serum samples.

Number of samples	53	Range of Samples	10 to 931 U/L
Slope	1.0081	y Intercept	-4.0906
Correlation Coefficient	0.9986	Regression Equation:	Y = 1.0081*X – 4.0906

** Cobas Mira is a registered trademark of Roche Diagnostics Operations, INC., Indianapolis, IN.*

Imprecision (CLSI, EP5-A2)

Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level U/L	Within Run SD U/L	Within Run CV %
500	4.3	0.9
191	1.6	0.9
96	1.1	1.1

Total Imprecision:

QC Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
500	10.8	2.2
191	4.7	2.5
96	1.7	1.7

Linearity (CLSI, EP6-A)
Linear from 7 to 1200 U/L, based on the linear regression Y = 0.9911*X + 17.661.

Limit of Blank (LOB):	1.73 U/L	(CLSI, EP17-A)
Limit of Detection (LOD):	2.38 U/L	(CLSI, EP17-A)
Limit of Quantitation (LOQ):	6.7 U/L	(CLSI, EP17-A, modified)

Interfering Substances (CLSI, EP7-A)

Less than 10% interference was classified as “no significant interference.”
There is significant interference at hemoglobin levels above 250 mg/dL. Avoid using hemolyzed serum or plasma samples.
No significant interference was found with levels up to 25 mg/dL of bilirubin.
No significant interference was found with levels up to 1000 mg/dL of triglycerides (using Intralipid*).

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[6 7]

REFERENCES

1 Quest, Vol 7(1), Feb 2000.
2 Szasz G. Proceedings of the Second International Symposium on Clinical Enzymology, Chicago October 1975.
3 Rosalki SB. J. Lab Clin Chem 23:646, 1977.
4 Tietz NW. Clinical Guide to laboratory Tests. WB Saunders and Co., Philadelphia, PA, (1995) p. 180.
5 Data on file at Medica.
6 Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.
7 Young, DS., Pestaner, L.C., Gibberman, V.; *Effects of drugs on clinical laboratory tests.* Clin Chem 21: 246D, 1975.

EasyRA Assay Parameters (CK)

Wavelength (nm)	340
Reaction Type	Enzyme (0)
Reaction Direction	Increase
Reagent Blank	No
Sample Blank	No
Max. first interval Abs. change	0.08
Reaction Time	5.2 min
Calibration interval (maximum)	N/A
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	8.0
Diluent volume (µl)	20
Reagent volume R1 (µl)	128
Reagent volume R2 (µl)	32
Decimal Places (default)	0
Units (default values)	U/L
Dilution Factor	1:1(to extend measuring range)
Linearity	7 to 1200 U/L
Molar Absorptivity	6.22

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