Application Sheet

cobas®

Laboratory Name

Test Name: Creatine Kinase

System information

For **cobas c** 311/501 analyzers:
CK2: ACN 550

Intended use

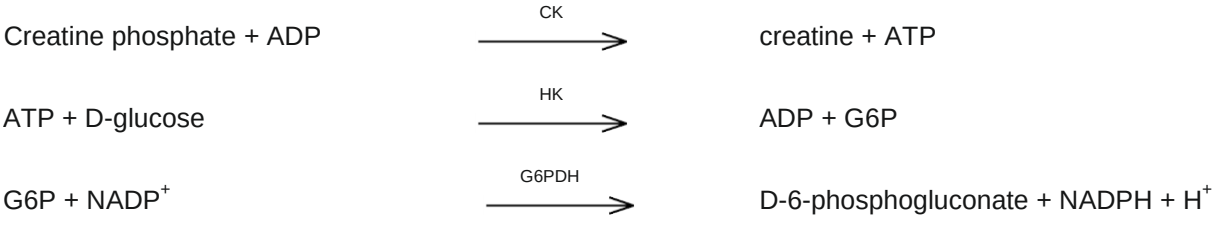
In vitro test for the quantitative determination of creatine kinase (CK) in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Creatine kinase (CK) is a dimeric enzyme occurring in four different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK-MM (skeletal muscle type), CK-BB (brain type) and CK-MB (myocardial type).[1]
The determination of CK and CK-isoenzyme activities is utilized in the diagnosis and monitoring of myocardial infarction and myopathies such as the progressive Duchenne muscular dystrophy. Following injury to the myocardium, such as occurs with acute myocardial infarction[1], CK is released from the damaged myocardial cells. In early cases, a rise in the CK-activity can be found just 4 hours after an infarction.[1,2] The CK activity reaches a maximum after 12-24 hours and then falls back to the normal range after 3-4 days.[1,2]
The assay method using creatine phosphate and ADP was first described by Oliver[3], modified by Rosalki[4] and further improved for optimal test conditions by Szasz et al.[5] CK is rapidly inactivated by oxidation of the sulfhydryl groups in the active center. The enzyme can be reactivated by the addition of acetylcysteine (NAC).[5] Interference by adenylate kinase is prevented by the addition of diadenosine pentaphosphate[6] and AMP.[5,6]
Standardized methods for the determination of CK with activation by NAC were recommended by the German Society for Clinical Chemistry (DGKC)[6] in 1977 and the International Federation of Clinical Chemistry (IFCC)[7] in 1991. In 2002 the IFCC confirmed their recommendation and extended it to 37 °C.[8,9]
The method described here is derived from the formulation recommended by the IFCC and was optimized for performance and stability.

Test principle

UV-test



Equimolar quantities of NADPH and ATP are formed at the same rate. The photometrically measured rate of formation of NADPH is directly proportional to the CK activity.

Reagents - working solutions

R1 Imidazole buffer: 123 mmol/L, pH 6.5 (37 °C); EDTA: 2.46 mmol/L; Mg^{2+}: 12.3 mmol/L; ADP: 2.46 mmol/L; AMP: 6.14 mmol/L; diadenosine pentaphosphate: 19 µmol/L; $NADP^{+}$ (yeast): 2.46 mmol/L; N-acetylcysteine: 24.6 mmol/L; HK (yeast): ≥ 36.7 µkat/L; G6PDH (E. coli): ≥ 23.4 µkat/L; preservative; stabilizers; additives.

R2 CAPSO* buffer: 20 mmol/L, pH 8.8 (37 °C); glucose: 120 mmol/L; EDTA: 2.46 mmol/L; creatine phosphate: 184 mmol/L; preservative; stabilizers.

*CAPSO: 3-(cyclohexylamine)-2-hydroxy-1-propanesulfonic acid

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
For USA: For prescription use only.
This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H360D	May damage the unborn child.

Prevention:

P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P308 + P313	IF exposed or concerned: Get medical advice/attention.

Storage:

P405	Store locked up.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling primarily follows EU GHS guidance.
Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

CK

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum: Nonhemolyzed serum is the specimen of choice and also recommended by IFCC.
Plasma: Li-heparin, K_2-, K_3-EDTA plasma.
Please note: Differences in the degree of hemolysis resulting from the blood sampling procedure used can lead to deviating results in serum and plasma.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.

Stability in serum:[10]	2 days at 20-25 °C
	7 days at 4-8 °C
	4 weeks at -20 °C
Stability in EDTA/heparin plasma:[11]	2 days at 15-25 °C
	7 days at 2-8 °C
	4 weeks at (-15)-(-25) °C

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section
General laboratory equipment
In addition, other suitable control material can be used.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 24-44		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.8 µL	–	–
Decreased	2.8 µL	15 µL	150 µL
Increased	2.8 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against the IFCC Method for Creatine Kinase.[8]

Quality control

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy
Refer to Brown Clinic Quatliy Control Specialty and Subspecialty Policy

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

Conversion factor:	U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a creatine kinase activity of 140 U/L (2.34 µkat/L).
Icterus:[12] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).
Hemolysis:[12] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62.1 µmol/L or 100 mg/dL). The level of interference may be variable depending on the exact content of erythrocytes.
Lipemia (Intralipid):[12] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Highly lipemic specimens

(L index > 1000) may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.
Drugs: No interference was found at therapeutic concentrations using common drug panels.[13,14]
Cyanokit (Hydroxocobalamin) at therapeutic concentrations interferes with the test.
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

7-2000 U/L (0.12-33.4 µkat/L)
Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:11 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 11.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 7 U/L (0.12 µkat/L)
Limit of Detection	= 7 U/L (0.12 µkat/L)
Limit of Quantitation	= 7 U/L (0.12 µkat/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.
The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the limit of blank with a probability of 95 %).
The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a precision of 20 % CV. It has been determined using low concentration creatine kinase samples.

Expected values

Reference intervals strongly depend on the patient group and the specific clinical situation.

For healthy people, according to Klein et al.:[16]

CK	U/L	µkat/L
Men	39-308	0.65-5.14
Women	26-192	0.43-3.21

Consensus values:[17]

CK	U/L	µkat/L
Men	< 190	< 3.20

Women	< 170	< 2.85

Consensus values:[17]

CK-MB	U/L	µkat/L
Men/women	< 25	< 0.42

Myocardial infarction: There is a high probability of myocardial damage when the following three conditions are fulfilled:[18]

		U/L	µkat/L
1	CK_{men}	> 190	> 3.17
	CK_{women}	> 167	> 2.79
2	CK-MB	> 24	> 0.40
3	The CK-MB activity accounts for 6-25 % of the total CK activity.		

According to Tietz:[19]

CK	U/L	µkat/L
Adult males > 19 years	20-200	0.33-3.34
Adult females > 19 years	20-180	0.33-3.01

The reference values according to Klein et al. are based on the 95th percentile of a group of healthy persons (202 men and 217 women) not involved in high-intensity athletic activities.
In order to ensure high sensitivity in the diagnosis of heart diseases the values given by Tietz are recommended. The loss of diagnostic specificity thereby incurred can be compensated for by additionally determining CK-MB and/or troponin T. When myocardial infarction is suspected the diagnostic strategy proposals in the consensus document of European and American cardiologists should in general be followed.[20]
If despite the suspicion of myocardial infarction the values found remain below the stated limits, a fresh infarction may be involved. In such cases, the determinations should be repeated after 4 hours.
CK varies with physical activity level and race in healthy individuals.[19,21]
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

References

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Alternative method

Refer to Brown Clinic Back-up Testing Policy

Source document

Reagent Name: CK
Method Sheet Version: V1.0 English

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
07190794 190	Creatine Kinase (200 tests)	System-ID 07 7485 5	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	

10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 × 5 mL)	Code 300	
10171735 122	Precinorm U (4 × 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
11447378 122	Precinorm CK-MB (4 x 3 mL)	Code 320	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

Effective date

Effective date for this procedure:

Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)^{cm} Date: 4/9/2018

Approved by: Date:

REVIEW – REVISION SUMMARY DOCUMENTATION

Date: By: Revision Summary: