

**CK****Creatine Kinase****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
07190794 190	Creatine Kinase (200 tests)	System-ID 07 7485 5
10759350 190	Calibrator f.a.s. (12 x 3 mL)	System-ID 07 3718 6
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	System-ID 07 3718 6
12149435 122	Precinorm U plus (10 x 3 mL)	System-ID 07 7999 7
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	System-ID 07 7999 7
12149443 122	Precipath U plus (10 x 3 mL)	System-ID 07 8000 6
12149443 160	Precipath U plus (10 x 3 mL, for USA)	System-ID 07 8000 6
10171743 122	Precinorm U (20 x 5 mL)	System-ID 07 7997 0
10171735 122	Precinorm U (4 x 5 mL)	System-ID 07 7997 0
10171778 122	Precipath U (20 x 5 mL)	System-ID 07 7998 9
10171760 122	Precipath U (4 x 5 mL)	System-ID 07 7998 9
11447378 122	Precinorm CK-MB (4 x 3 mL)	System-ID 07 9111 3
04358210 190	Precipath CK-MB (4 x 3 mL, not available in the USA)	System-ID 07 6828 6
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	System-ID 07 7469 3
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	System-ID 07 7469 3
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	System-ID 07 7469 3
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	System-ID 07 7470 7
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	System-ID 07 7470 7
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	System-ID 07 7470 7
20756350 322	NaCl Diluent 9 % (6 x 22 mL)	System-ID 07 5635 0

**English****System information**

Test CK2, test ID 0-045

**Intended use**

In vitro test for the quantitative determination of creatine kinase (CK) in human serum and plasma on COBAS INTEGRA 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).<sup>1</sup>

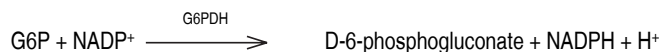
The determination of CK and CK-isoenzyme activities is utilized in the diagnosis and monitoring of myocardial infarction and myopathies such as the progressive Duchenne muscular dystrophy. Following injury to the myocardium, such as occurs with acute myocardial infarction<sup>1</sup>, CK is released from the damaged myocardial cells. In early cases, a rise in the CK activity can be found just 4 hours after an infarction.<sup>1,2</sup> The CK-activity reaches a maximum after 12-24 hours and then falls back to the normal range after 3-4 days.<sup>1,2</sup>

The assay method using creatine phosphate and ADP was first described by Oliver<sup>3</sup>, modified by Rosalki<sup>4</sup> and further improved for optimal test conditions by Szasz et al.<sup>5</sup> 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).<sup>5</sup> Interference by adenylate kinase is prevented by the addition of diadenosine pentaphosphate<sup>6</sup> and AMP.<sup>5,6</sup>

Standardized methods for the determination of CK with activation by NAC were recommended by the German Society for Clinical Chemistry (DGKC)<sup>6</sup> in 1977 and the International Federation of Clinical Chemistry (IFCC)<sup>7</sup> in 1991. In 2002 the IFCC confirmed their recommendation and extended it to 37 °C.<sup>8,9</sup> 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<sup>2+</sup>: 12.3 mmol/L; ADP: 2.46 mmol/L; AMP: 6.14 mmol/L; diadenosine pentaphosphate: 19 μmol/L; NADP<sup>+</sup> (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.

**SR** 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 SR is in position C.

**Precautions and warnings**

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

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.

**CK****Creatine Kinase****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**

Shelf life at 2-8 °C	See expiration date on <b>cobas c</b> pack label
On-board in use at 10-15 °C	8 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<sub>2</sub>-, K<sub>3</sub>-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: <sup>10</sup>	2 days at 20-25 °C
	7 days at 4-8 °C
	4 weeks at -20 °C
Stability in EDTA/heparin plasma: <sup>11</sup>	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)**

NaCl Diluent 9 %, Cat. No. 20756350322, system-ID 07 5635 0 for automatic sample dilution. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus analyzers.

**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.

**Application for serum and plasma****COBAS INTEGRA 400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Kinsearch
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/552 nm
Calc. first/last	10/45-62
Unit	U/L

**Pipetting parameters**

		Diluent (H <sub>2</sub> O)
R1	100 µL	-
Sample	2.75 µL	2 µL
SR	20 µL	-
Total volume	124.75 µL	

**Calibration**

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized against the IFCC Method for Creatine Kinase.<sup>8</sup>

**Quality control**

Reference range	Precinorm U, Precinorm U plus, Precinorm CK-MB or PreciControl ClinChem Multi 1
Pathological range	Precipath U, Precipath U plus, Precipath CK-MB* or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

\*Not for use in the US

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

**Calculation**

COBAS INTEGRA analyzers automatically calculate the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus analyzer).

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

**Limitations - interference**

Criterion: Recovery within  $\pm 10\%$  of initial value at a creatine kinase activity of 140 U/L (2.34  $\mu\text{kat/L}$ ).

Icterus:<sup>12</sup> No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026  $\mu\text{mol/L}$  or 60 mg/dL).

Hemolysis:<sup>12</sup> No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62.1  $\mu\text{mol/L}$  or 100 mg/dL). The level of interference may be variable depending on the exact content of erythrocytes.

Lipemia (Intralipid):<sup>12</sup> 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.<sup>13,14</sup>

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.<sup>15</sup>

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 COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

**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  $\mu\text{kat/L}$ )

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  $\mu\text{kat/L}$ )

Limit of Detection = 7 U/L (0.12  $\mu\text{kat/L}$ )

Limit of Quantitation = 7 U/L (0.12  $\mu\text{kat/L}$ )

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 95<sup>th</sup> percentile value from  $n \geq 60$  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.:<sup>16</sup>

CK	U/L	$\mu\text{kat/L}$
Men	39-308	0.65-5.14
Women	26-192	0.43-3.21

Consensus values:<sup>17</sup>

CK	U/L	$\mu\text{kat/L}$
Men	< 190	< 3.20
Women	< 170	< 2.85
Consensus values: <sup>17</sup>		
CK-MB	U/L	$\mu\text{kat/L}$
Men/women	< 25	< 0.42

Myocardial infarction: There is a high probability of myocardial damage when the following three conditions are fulfilled:<sup>18</sup>

	U/L	$\mu\text{kat/L}$
1 CK <sub>men</sub>	> 190	> 3.17
CK <sub>women</sub>	> 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:<sup>19</sup>

CK	U/L	$\mu\text{kat/L}$
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 95<sup>th</sup> 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.<sup>20</sup>

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.<sup>19,21</sup>

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**

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

**Precision**

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	Mean U/L ( $\mu\text{kat/L}$ )	SD U/L ( $\mu\text{kat/L}$ )	CV %
Human serum 1	22.1 (0.37)	0.9 (0.01)	3.9
Human serum 2	144 (2.40)	1.4 (0.02)	1.0
Human serum 3	494 (8.25)	4.6 (0.08)	0.9
Human serum 4	980 (16.4)	10 (0.2)	1.0
Human serum 5	1893 (31.6)	19 (0.3)	1.0
PCCC Multi 1*	162 (2.71)	1.5 (0.03)	0.9
PCCC Multi 2	311 (5.19)	2.9 (0.05)	0.9

Intermediate precision	Mean U/L ( $\mu\text{kat/L}$ )	SD U/L ( $\mu\text{kat/L}$ )	CV %
Human serum 1	22.2 (0.37)	1.0 (0.02)	4.6

Intermediate precision	Mean U/L (μkat/L)	SD U/L (μkat/L)	CV %
Human serum 2	145 (2.42)	1.9 (0.03)	1.3
Human serum 3	498 (8.31)	5.7 (0.10)	1.1
Human serum 4	980 (16.4)	12 (0.19)	1.2
Human serum 5	1893 (31.6)	22 (0.36)	1.1
PCCC Multi 1*	161 (2.69)	2.0 (0.03)	1.3
PCCC Multi 2	309 (5.16)	3.6 (0.06)	1.2

\*PCCC = PrediControl ClinChem

**Method comparison**

Creatine kinase values for human serum and plasma samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined using the CKL reagent on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 109

Passing/Bablok <sup>22</sup>	Linear regression
$y = 0.999x + 12.5 \text{ U/L}$	$y = 0.987x + 19.7 \text{ U/L}$
$r = 0.980$	$r = 0.999$

The sample activities were between 11.7 and 1819 U/L (0.20 and 30.4 μkat/L).

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

**FOR US CUSTOMERS ONLY: LIMITED WARRANTY**

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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