FRA

cobas®

Fructosamine

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08105677190	08105677500	Fructosamine (550 tests)	System-ID 2058 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

11098993122	Precimat Fructosamine (3 x 1 mL)	Code 20581	
11098985122	Precinorm Fructosamine (3 x 1 mL)	Code 20321	
11174118122	Precipath Fructosamine (3 x 1 mL)	Code 20322	

English

System information
FRA: ACN 20580

Intended use
In vitro test for the quantitative determination of glycated proteins (fructosamine) in human serum and plasma on **cobas c** systems.

Summary
Fructosamine measurements performed with this assay in human serum and plasma can be used as an aid in the assessment of diabetes mellitus.

Fructosamine is formed by the non-enzymatic reaction (glycation) between a sugar (glucose or fructose) and the amino acid group of a serum protein (primarily albumin), therefore forming a ketoamine.[1] The formation of fructosamine is a two-step reaction, as a first step a Schiff base is formed by the reversible coupling of glucose and other sugars to a serum protein which, in a second step, is transformed by non-reversible Amadori rearrangement to the corresponding ketoamine (fructosamine). The formation of fructosamine increases with the level of blood glucose.[2] Metabolization occurs within 1 to 3 weeks, corresponding to the turnover of most serum proteins. The concentration of fructosamine thus reflects the average of the continuously varying blood glucose concentrations during this period, serving as a blood glucose memory.[2,3,4] Fructosamine can therefore be used as an indicator of glycemia in the assessment of diabetes mellitus, in particular in conjunction with glycated hemoglobin (HbA1c) or when measurement of HbA1c is invalid for monitoring of diabetes control.[5,6,7]

Test principle
Colorimetric test by reaction with nitroblue tetrazolium.[8,9,10]

The colorimetric test for fructosamine (glycated protein) is based on the ability of ketoamines to reduce nitroblue tetrazolium in alkaline medium. The rate of formation of formazan is directly proportional to the fructosamine concentration and is measured photometrically.

Reagents - working solutions

R1 Nitroblue tetrazolium: 1.2 mmol/L; uricase (microbial): ≥ 12 µkat/L; pH 7.5; non-reactive buffer; stabilizer; surfactants

R3 Carbonate buffer: 1.5 mol/L; pH 10.4

R1 is in position B and R3 is in position C.

Precautions and warnings
For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H315	Causes skin irritation.
H318	Causes serious eye damage.

Prevention:

P264	Wash skin thoroughly after handling.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P302 + P352	IF ON SKIN: Wash with plenty of water.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
P332 + P313	If skin irritation occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Product safety labeling follows EU GHS guidance.
Contact phone: all countries: +49-621-7590

Reagent handling
Ready for use

Storage and stability

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

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: Collect serum using standard sampling tubes.
Plasma: Li-heparin and K_2-EDTA 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.

See the limitations and interferences section for details about possible sample interferences.

Stability:	3 days at 15-25 °C[11]
	2 weeks at 2-8 °C[11]
	2 months at (-15)-(-25) °C[12]

Freeze only once.

Materials provided
See “Reagents – working solutions” section for reagents.

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Materials required (but not provided)

See “Order information” section

General laboratory equipment

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/546 nm		
Reagent pipetting		Diluent (H_2O)	
R1	45 µL	21 µL	
R3	9 µL	15 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (H_2O)*
Normal	4.5 µL	–	–
Decreased	2.3 µL	–	–
Increased	4.5 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: Precimat Fructosamine
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against fructose polylysine standard.

Quality control

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 8 weeks.

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 c systems automatically calculate the analyte concentration of each sample in the unit µmol/L.

Limitations – interference

Criterion: Recovery within ± 29 µmol/L of initial values of samples ≤ 285 µmol/L and within ± 10 % for samples > 285 µmol/L.

Icterus:[13] No significant interference up to an I index of 4 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 68 µmol/L or 4 mg/dL).

Hemolysis:[13] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 100 mg/dL).

Lipemia:[13] No significant interference up to an L index of 1800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

Exception: Levodopa causes artificially high fructosamine results. Oxytetracycline causes artificially high fructosamine results.

As tested according to CLSI recommendation methyldopa causes artificially high fructosamine results.[16]

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 99.4 µmol/L (17.5 mg/L).

In hydremic states (pregnancy for instance) it may be favorable to relate fructosamine to protein using the following formula:

$$\text{Fructosamine}_{corr} = \frac{\text{measured fructosamine} \times 72}{\text{measured total protein (in g/L)}}$$

Dysproteinemic states may affect fructosamine values.[17]

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[18]

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 c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator’s manual.

Limits and ranges

Measuring range

14-1000 µmol/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 14 µmol/L
Limit of Detection	= 14 µmol/L
Limit of Quantitation	= 14 µmol/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 total error of 20 %. It has been determined using low concentration fructosamine samples.

Expected values[9,19]

Fructosamine concentrations were determined in 555 apparently healthy subjects between the ages of 20 and 60. A reference range of 205 to 285 µmol/L was determined in this study for adults without diabetes. In a poorly controlled diabetic population, mean fructosamine values were reported to be 396 µmol/L (range 228-563 µmol/L). A fructosamine concentration above the established expected value is an indicator for hyperglycemia during the preceeding 1-3 weeks or longer.

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 analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean µmol/L*	*SD µmol/L*	*CV %*
Precinorm Fructosamine	270	1.81	0.7
Precipath Fructosamine	527	3.07	0.6
Human serum 1	45.5	1.64	3.6
Human serum 2	109	1.30	1.2
Human serum 3	299	1.73	0.6
Human serum 4	526	3.63	0.7
Human serum 5	937	4.53	0.5

Intermediate precision	*Mean µmol/L*	*SD µmol/L*	*CV %*
Precinorm Fructosamine	269	3.10	1.1
Precipath Fructosamine	526	6.16	1.2
Human serum 1	45.5	1.67	3.7
Human serum 2	109	1.63	1.5
Human serum 3	302	2.25	0.7
Human serum 4	526	5.30	1.0
Human serum 5	937	8.55	0.9

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Fructosamine values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 75

Passing/Bablok[20]	Linear regression
y = 0.976x + 19.6 µmol/L	y = 0.972x + 19.8 µmol/L
τ = 0.920	r = 0.998

The sample concentrations were between 19.1 and 960 µmol/L.

Fructosamine values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 67

Passing/Bablok[20]	Linear regression
y = 1.015x + 13.0 µmol/L	y = 1.019x + 11.7 µmol/L
τ = 0.949	r = 0.998

The sample concentrations were between 27.9 and 969 µmol/L.

Fructosamine values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 68

Passing/Bablok[20]	Linear regression
y = 0.990x - 0.825 µmol/L	y = 0.991x - 0.163 µmol/L
τ = 0.979	r = 1.000

The sample concentrations were between 26.4 and 989 µmol/L.

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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 for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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