FRA

cobas®

Fructosamine

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04537939190	04537939500	Fructosamine (150 tests)	System-ID 07 3756 9	**cobas c** 311, **cobas c** 501/502, COBAS INTEGRA 400 plus

Materials required (but not provided):

		cobas c 311, **cobas c** 501/502	COBAS INTEGRA 400 plus
11098993122	Precimat Fructosamine (3 x 1 mL)	Code 581	System-ID 07 9171 7
11098985122	Precinorm Fructosamine (3 x 1 mL)	Code 321	System-ID 07 9164 4
11174118122	Precipath Fructosamine (3 x 1 mL)	Code 322	System-ID 07 9170 9

English

Intended use

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

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

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.

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.

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Calculation

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

Expected values[9,13]

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.

cobas c systems

System information

For **cobas c** 311/501 analyzers:

FRA: ACN 667

For **cobas c** 502 analyzer:

FRA: ACN 8667

Reagents - working solutions

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

R2 Carbonate buffer: 1.5 mol/L; pH 10.4

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

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

Application for serum and plasma

cobas c 311 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 52-57	
Wavelength (sub/main)	700/546 nm	
Reaction direction	Increase	
Unit	µmol/L	
Reagent pipetting		Diluent (H_2O)
R1	60 µL	28 µL
R2	12 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	6 µL	–	–
Decreased	3 µL	–	–
Increased	6 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 63-70	
Wavelength (sub/main)	700/546 nm	
Reaction direction	Increase	
Unit	µmol/L	
Reagent pipetting		Diluent (H_2O)
R1	60 µL	28 µL
R2	12 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	6 µL	–	–
Decreased	3 µL	–	–
Increased	6 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A	
Reaction time / Assay points	10 / 63-70	
Wavelength (sub/main)	700/546 nm	
Reaction direction	Increase	
Unit	µmol/L	
Reagent pipetting		Diluent (H_2O)
R1	60 µL	28 µL
R2	12 µL	20 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	6 µL	–	–
Decreased	3 µL	–	–
Increased	12 µL	–	–

Calibration

Calibrators	S1: H_2O S2: Precimat Fructosamine
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • 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. 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.

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:[14] 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:[14] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 100 mg/dL).

Lipemia:[14] 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.[15,16]

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

As tested according CLSI recommendation Methyldopa causes artificially high fructosamine results.[17]

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.[18]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[19]

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. 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 required in certain cases.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

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

Lower detection limit of the test

14 µmol/L

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Specific performance data

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

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained on the **cobas c** 501 analyzer:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
Precinorm Fructosamine	262	4	1.6
Precipath Fructosamine	498	4	0.7
Human serum 1	262	2	0.9
Human serum 2	208	2	1.0
Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
Precinorm Fructosamine	262	4	1.5
Precipath Fructosamine	489	6	1.2
Human serum 3	266	4	1.5
Human serum 4	210	4	1.8

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

Fructosamine values for human serum and plasma samples obtained on a **cobas c** 501 analyzer (y) were compared with those determined on Roche/Hitachi 917/MODULAR P analyzers (x), using the corresponding reagent.

Sample size (n) = 231

Passing/Bablok[20]	Linear regression
y = 0.968x + 15.0 µmol/L	y = 0.967x + 15.5 µmol/L
τ = 0.946	r = 0.998

The sample concentrations were between 166 and 836 µmol/L.

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

COBAS INTEGRA systems

System information

FRA: Test ID 0-056.

Reagents - working solutions

R1	Nitroblue tetrazolium: 1.2 mmol/L; uricase (microbial): ≥ 12 µkat/L; pH 7.5; non-reactive buffer; stabilizer; surfactants
SR	Carbonate buffer: 1.5 mol/L; pH 10.4

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

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	8 weeks

Application for serum and plasma

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/652 nm
Calc. first/last	86/98
Unit	µmol/L

Fructosamine is measured as long analysis test (duration approximately 17 minutes).

Pipetting parameters

		Diluent (H_2O)
R1	60 µL	24 µL
Sample	6 µL	12 µL
SR	12 µL	12 µL
Total volume	126 µL	

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Calibration

Calibrator	Precimat Fructosamine Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration frequency:	After reagent lot change and as required following quality control

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

Reference range	Precinorm Fructosamine
Pathological range	Precipath Fructosamine
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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.

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:[14] No significant interference up to an I index of 5 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 85 µmol/L or 5 mg/dL).

Hemolysis:[14] No significant interference up to an H index of 50 (approximate hemoglobin concentration: 31 µmol/L or 50 mg/dL).

Lipemia (Intralipid):[14] No significant interference up to an L index of 2000. 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.[15,16]

Exception: levodopa, methyldopa, calcium dobesilate, and oxytetracycline cause artificially high fructosamine values.

Physiological ascorbic acid levels do not interfere with the fructosamine test. Ascorbic acid levels higher than 227 µmol/L (4 mg/dL) interfere with the test significantly. No significant interference up to a glucose level of 45 mmol/L (810 mg/dL).

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.[18]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[19]

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

14-900 µmol/L

Lower detection limit

Lower detection limit of the test:
14 µmol/L

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 30).

Specific performance data

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

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained on the COBAS INTEGRA 700 analyzer:

	Level 1	Level 2
Mean	181 µmol/L	450 µmol/L
CV repeatability	0.92 %	0.65 %
CV intermediate precision	2.8 %	2.5 %

The data obtained on COBAS INTEGRA 700 analyzer(s) are representative for COBAS INTEGRA 400 analyzer(s).

Method comparison

Fructosamine values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Fructosamine reagent (y) were compared with those determined using the commercially available reagents for fructosamine on a COBAS MIRA system and an alternative manufacturer's clinical chemistry system (x). Samples were measured in duplicate. Sample size (n) represents all replicates.

		COBAS MIRA system	Alternative system
Sample size	(n)	148	200
Corr. coefficient	(r)	0.993	0.995
	(r_s)	0.990	0.991
Linear regression		y = 1.06x - 10 µmol/L	y = 0.99x - 13 µmol/L
Passing/Bablok[20]		y = 1.07x - 15 µmol/L	y = 0.98x - 11 µmol/L

The sample concentrations were between 73 and 495 µmol/L.

The data obtained on COBAS INTEGRA 700 analyzer(s) are representative for COBAS INTEGRA 400 analyzer(s).

References

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2 Danese E, Montagnana M, Nouvenne A et al. Advantages and pitfalls of fructosamine and glycated albumin in the diagnosis and treatment of diabetes. J Diabetes Sci Technol 2015 Mar;9(2):169-176.

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7 National Institute for Health and Care Excellence (NICE) (2015). Type 2 diabetes in adults: management (NICE Guideline [NG28]) [revised 2022 June; cited 2023 December 4]. Available from: https://www.nice.org.uk/guidance/ng28

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20 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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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