

FRUCTOSAMINE

COLORIMETRIC DETERMINATION IN SERUM AND PLASMA MODIFIED NBT METHOD
 Only for in vitro diagnostic use

Kit: 10 x 10 ml

Cod. FR289

SUMMARY

Fructosamine is a glycosylated protein that is originated by a non-enzymatic reaction of glucose with amino groups of extracellular proteins. An excess of fructosamine in blood indicates complications of diabetes mellitus. The dosages of fructosamine associated with those of glycemia, glycosylated haemoglobin is useful for monitoring diabetes. When the proteinic metabolism is altered, the dosage of fructosamine can give false elevated values.

PRINCIPLE

At alkaline pH, the glucose linked at amminic groups of proteins with stable ketoaminc link (fructosamine) reduce the blue of nitrotetrazolium. The coloured intensity of complex formed is proportional at the concentration of fructosamine in the sample.

REAGENTS

Components of the kit: **Code FR289**
***REAGENT 1 (liquid)** **2 x 50 ml**
 Carbonate buffer 0.2 N
***REAGENT 2 (powder)** **10 x 10 ml**
 NBT >0,1 mmol/L
 Uricase >1000 U/L

STABILITY: the reagents, stored at 2-8°C, are stable up to the expire date shown on the package **if not contaminated during handling.**

AUXILIARY REAGENTS FOR CALIBRATION and for QUALITY CONTROL (Not supplied with the kit)

We suggest strongly to calibrate always on the instruments.

To grant a good calibration we suggest to use following kit:

- CALIBRATOR **Cod. CALFRU**

To grant the correct test performances we suggest to use following kits:

- NORMAL CONTROL **Cod. FRUNOR**

- PATHOLOGICAL CONTROL **Cod. FRUPAT**

PREPARATION OF WORKING REAGENT

Add 10 ml of *Reagent 1 to one vial of *Reagent 2.
 Mix gently until complete dissolution.
 The working solution is ready after 10 minutes from reconstitution.

Mix kindly before use.

Close immediately after handling. The Reagents have to be used correctly, to avoid contamination.

Incompetent handling will release us from any responsibility.

STABILITY: 5 days at 2-8°C, 8 hours at room temp. in the dark, **if not contaminated during handling.**

SAMPLE

• Not haemolyzed serum, EDTA or heparin plasma.

PROCEDURE

- Wavelength: 550 nm
- Pathlength: 1 cm
- Reading: against Reagent Blank
- Temperature: 37°C.
- Method: fixed time
- Reaction: 10 + 5 minutes
- Linearity: up to 1000 µmol/L
- Sample/Reagent: 1/20

Let the reagent reach the working temperature before use.

Pipette in a test tube or cuvette so labelled:

R/B: Reag. Blank; ST: Standard/Calibrator; S: Sample:

	R/B	S	ST
Working Reag.	1000 µl	1000 µl	1000 µl
Sample	----	50 µl	----
Standard	----	----	50 µl
Distilled water	50 µl	----	----

Mix wells, incubate for 10 minutes at 37°C. Read the absorbance of standard (Ast1) and sample (As1) against Blank reagent. Exactly after 5 minutes at 37°C read again standard (Ast2) and sample (As2).; against the Reagent Blank.

Determine the diff. of absorbance for sample and calibrator:

$$\Delta As = A2s - A1s$$

$$\Delta Ast = A2st - A1st$$

CALCULATION

$(\Delta As / \Delta Ast) \times \text{Calibrator conc.} = \mu\text{mol/L fructosamine.}$

REFERENCE VALUES

FRUCTOSAMINE:

No diabetics: up to 285 µmol/L.

It is suitable that every laboratory determines its reference values

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The concentration of fructosamine is determined between 5 - 1000 µmol/L
 For concentration of fructosamine higher than 1000 µmol/L dilute the sample 1:2 with saline solution, repeat the determination and multiply the result x 2.

Sensitivity: The minimum detectable is 5 µmol/L

Within-run Precision:

	Mean (µmol/L) ± 2s	CV %
Serum 1	168,90 ± 5,84	1,73
Serum 2	655,30 ± 13,12	1,00

Run-to-run (Day-to-day) Precision:

	Mean (µmol/L) ± 2s	CV %
Serum 1	174,90 ± 13,23	3,78
Serum 2	682,53 ± 56,94	4,17

Interferences: See References point 2.

Correlation: A group of 20 sera was assayed by this procedure and using a similar commercially available Fructosamine Reagent. Comparison of the data gave following results

Linear regression $Y = 1,0160X - 5,3$
 $r = 0,9982$

NOTE

1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concentration of fructosamine higher than 1000 $\mu\text{mol/L}$ dilute the sample 1:2 with saline solution, repeat the determination and multiply the result $\times 2$.
4. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
5. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Fructosamine (see References 2.).
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.
8. An eventual coloration of working solution do not influence the result of the determination.
9. Uric ac. and lipemia do not interfere with the test; the bilirubin up to 2 mg/dL, the hemoglobin up to 100 mg/dL and the ascorbic acid up to 3 mg/dL, do not interfere.
10. Altered states of proteic metabolism can influence the determination of fructosamine.
11. In hydremic states (ex. pregnancy) can be useful put in relation the fructosamine with proteins, using the formula:

$$\text{Fructosamine rel. } (\mu\text{mol/L}) = \frac{\text{value det. of fructosamine} \times 7,2}{\text{value det. of tot. protein (in g/dL)}}$$

12. The kit Fructosamine calibrator is a primate product and has to be used as a potential transfer of infective pathologies. This product has been tested and found to be negative for HIV, HCV and HBsAg antibodies by an approved method. Because no test method can offer complete assurance that all infectious agents are absent, it is recommended that this product and all the samples be handled as though capable of transmitting infectious disease.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).
3. Johnson R.N., et al., Clin. Chim. Acta 127, 87 (1983).
4. Schleicher E. D. et al., Clin. Chem. 36, 136 (1990).

Ver. 2004/10