# Fructosamine Reagent

| Order No. | Description |
|---|---|
| R85317 | 5 x 20 mL, with Calibrator |

## INTENDED USE

These reagents are intended for the quantitative in vitro measurement of fructosamine in human serum.

Measurement of fructosamine indicates the degree of glycemic control in diabetic patients over a short term (2 to 3 weeks).

## CLINICAL SIGNIFICANCE

Elevated levels of blood glucose are held responsible for many case complications of diabetes. Glucose reacts non-enzymatically with proteins to form unstable Schiff's bases which, through an Amadori conversion, are rearranged into stable ketoamines (1).

Fructosamine indicates the ketoamine group formed by glucose with serum protein, and mainly albumin (2). The turnover rate of circulating proteins is approximately 2 to 3 weeks, thus the level of fructosamine is related to the average glucose concen-tration in blood over this period of time.

Glycosylated hemoglobin is formed by the same non-enzymatic type of reaction between glucose and hemoglobin, and its measurement has been accepted for several years in the management of the diabetic patient (3, 4). However, this assay reflects a blood glucose average over a period of 2 to 3 months, while fructosamine levels refer to a much shorter period of 2 to 3 weeks (5).

Fructosamine assays are also easier to perform than are those for glycohemoglobin.

While glucose levels may vary continuously, fructosamine (and glycohemoglobin) levels provide more meaningful data and appear to be true indicators of diabetic control.

The higher the fructosamine value, the poorer the degree of glycemic control (6, 7).

Measurements of fructosamine are a useful supplement to standard tests to screen for diabetes mellitus.

Fructosamine has been found to be a good indicator of gestational diabetes (8).

## TEST SUMMARY

This test method is based on the ability of the ketoamine group of glycated proteins to reduce a tetrazolium salt (INT) under alkaline conditions. The colorimetric endpoint reaction is measured at 500 nm.

Interference from endogenous ascorbic acid in the serum sample is eliminated by pre-treating the sample with an alkaline buffer solution. The pre-treated sample is then added to the reagent and the reaction is allowed to proceed to completion. The color of the formazan developed by the reduced tetrazolium salt is read colorimetrically at 500 nm. The concentration of the fructosamine in the sample is proportional to the absorbance of the formazan.

## REAGENTS COMPOSITION

**Fructosamine Diluent**

Non-reactive ingredients:

Buffers, stabilizers and fillers

**Fructosamine Reagent**

Reactive ingredients:

| | |
|---|---|
| p-iodonitrotetrazolium violet (INT) | 0.57 mmol/L |

Non-reactive ingredients:

Buffers, stabilizers and fillers

**Fructosamine Buffer**

Non-reactive ingredients:

Buffers, stabilizers and fillers

**Calibrator**

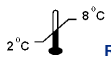
Non-reactive ingredients:

Buffers, stabilizers and fillers

Pooled human serum

## REAGENTS PREPARATION

1. Fructosamine Diluent
   This reagent is in liquid form ready to use.
2. Fructosamine Reagent
   Dissolve the contents of each vial with the volume of Carbonate Buffer specified on the vial label. Store the reconstituted reagent in the original amber glass vial in the refrigerator at 2–8 °C.
3. Fructosamine Buffer
   The Carbonate Buffer is in liquid form ready to use.
4. Calibrator
   Dissolve the contents of each vial with the volume of distilled or deionized water specified on the vial label. Cap vial and swirl gently to dissolve. Store the reconstituted calibrator in the refrigerator (2–8 °C).




## REAGENTS STORAGE AND STABILITY

1. The Fructosamine Diluent is stable until the label expiration date when stored at 2–25 °C.
2. The Fructosamine Reagent in the unopened vial is stable until the label expiration date when stored at 2–8 °C. The reconstituted reagent is stable for 30 days stored in the original amber glass vial at 18–25 °C or for 60 days stored at 2–8 °C. Protect reconstituted reagent from strong, direct light. Do not store the reagent, unreconstituted or reconstituted in the freezer.
3. The Fructosamine Buffer is stable until the label expiration date when stored at 2–25 °C.
4. The Calibrator in the unopened vial is stable until the label expiration date stored at 2–8 °C. After reconstitution the Calibrator is stable for 14 days at 2–8 °C. For assay use, remove a suitable aliquot of calibrator for pretreatment as directed; cap and refrigerate the vial.
5. The Cliniqa Fructosamine Reagents are for in vitro diagnostic use only. Storage conditions different from those recommended above may cause erroneous results.
6. Do not use the freshly reconstituted Fructosamine Reagent if it has an absorbance higher than 0.800 measured at 500 nm against a blank of water.

## PRECAUTIONS

Human serum was used in the manufacture of this product. Each donor unit was tested with licensed reagents and found negative for HBsAg and HCV and nonreactive for HIV antibody. Since no test method can assure that products derived from human blood do not contain HIV and Hepatitis B and Hepatitis C viruses, this material and all patient samples should be handled as though capable of transmitting infectious diseases.

Harmful by inhalation, in contact with skin, and if swallowed. Irritating to eyes, respiratory system and skin.

Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water for at least 10 minutes. If swallowed, seek medical advice immediately and show this container or label.

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

## SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum, separated from the cells as soon as possible after collection, is the specimen to be used in this assay. Avoid hemolysis and contamination of the sample with hemoglobin. Hemoglobin contamination in the sample greater than 0.25 g/dL can interfere with the assay. The glycated hemoglobin will react in the same manner as fructosamine.

Serum fructosamine concentrations are stable for at least 7 days refrigerated (2–8 °C). Samples stored in the freezer are stable for 3 months at -20 °C and up to 6 months at -90 °C. Do not freeze and thaw samples repeatedly.

## PRE-TREATMENT OF SAMPLE AND CALIBRATOR

The serum sample and calibrator must be pre-treated before assay. Add 10 µL of the Fructosamine Diluent (Cat. No. R85318VI) to 100 µL of serum or reconstituted calibrator. Mix, and allow to incubate at room temperature (20–25 °C) for at least 30 minutes.

If the assay is not performed within 60 minutes after the incubation period, refrigerate (2 – 8 °C) the treated sample and/or calibrator for up to 2 hours.

Incubation time for the treated samples and calibrator may be decreased by incubating at higher temperatures:

| Temperature | Incubation Time |
|---|---|
| 37 °C | 5 minutes |
| 45 °C | 3 minutes |

## INTERFERING SUBSTANCES

1. Bilirubin in the samples up to 40 mg/dL does not interfere in the assay.
2. Triglycerides concentrations in the samples up to 600 mg/dL will be corrected by the blanking technique used in the assay. Serum samples with triglycerides concentrations greater than 600 mg/dL should not be used.
3. Hemoglobin in the samples in concentrations less than 0.25 g/dL does not interfere with the assay.
4. The interference by normal concentrations of reducing substances such as ascorbic acid, glutathione and unbound sugars are eliminated by pre-treatment of the sample prior to assay.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer or colorimeter capable of accurate absorbance measurements at 500 nm.
2. Matched cuvettes, preferably square,1cm light path.
3. Test tubes or suitable containers for incubating reaction mixture.
4. Pipette to accurately measure water, reagents, and samples.

## MATERIALS PROVIDED

1. Fructosamine Diluent, in liquid form, 1 x 10 mL bottle, Cat. No. R85318VI.
2. Fructosamine Reagent, in dry powder form, 5 x 20 mL vials, Cat No. R85320VI.
3. Fructosamine Buffer, in liquid form, 2 x 125 mL bottles, Cat. No. R85319VI.
4. Calibrator, lyophilized, single concentration, 3 x 2 mL vials. The concentration of the calibrator is indicated on the vial label. Cat. No. R85321VI.

## TEST PROCEDURE, MANUAL METHOD

The described assay procedure is for manual use. The assay can be performed more conveniently and with increased precision on many automated and computerized analyzers.

Set up assay as follows:

A blank is to be performed with each sample, calibrator and control.

1. In separate cuvettes, or other suitable containers for incubating reaction mixtures, prepare blanks and tests respectively as indicated for Calibrator, Controls and Samples.
2. At timed intervals add 50 µL of the pre-treated Calibrator, Controls, and Samples to their respective cuvettes. The timing of the test sequence is essential; use an appropriate timing device.

32937_01 11/24/09

| Calibrator | Controls | | Sample | | | |
|---|---|---|---|---|---|---|
| | Blank | Test | Blank | Test | Blank | Test |
| Fructosamine Rgt. | — | 1 mL | — | 1 mL | — | 1 mL |
| Fructosamine Buffer | 1 mL | — | 1 mL | — | 1 mL | — |
| Calibrator | 50 µL | 50 µL | — | — | — | — |
| Controls | — | — | 50 µL | 50 µL | — | — |
| Sample | — | — | — | — | 50 µL | 50 µL |

3. Cover mouth of cuvette with Parafilm and mix by gentle inversion. Incubate for exact time period for selected temperature:

| Temperature | Incubate Exactly |
|---|---|
| Room Temperature (20–25 °C) | 15 minutes |
| 30 °C | 5 minutes |
| 37 °C | 3 minutes |

4. Set spectrophotometer to 500 nm and zero against water.
5. At the end of the selected temperature and time period, read and record the absorbance for each test and its respective blank. Read Test and Blank in the same set-up timed intervals to maintain identical timing and conditions.
6. Calculate the corrected absorbance value $A_c$ for Calibrator, Controls, and Samples by subtracting the blank absorbance value ($A_b$) from the test absorbance value ($A_t$):

$$A_c = A_t - A_b$$

## CALIBRATION

This assay requires the use of a Fructosamine calibrator. Use the calibrator provided with this reagent as directed. The use of fructosamine calibrators from other sources may not produce accurate results.

## CALCULATIONS

$$\frac{A_c \text{ Sample}}{A_c \text{ Calibrator}} \times \text{Conc. of Calibrator} = \text{mmol/L fructosamine in the sample.}$$

Sample Calculation:

If: $A_c$ of Sample = 0.111

$A_c$ of Calibrator = 0.166

Concentration of Calibrator = 3.44 mmol/L

Then:

$$\frac{0.111}{0.166} \times 3.44 \text{ mmol/L} = 2.30 \text{ mmol/L fructosamine in the sample.}$$

## QUALITY CONTROL

Controls are recommended for monitoring the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents, and techniques. Cliniqa Fructosamine Serum Controls I and II (Cat. No. R85393) are recommended for this purpose.

## LIMITATIONS OF THE PROCEDURE

The present method measures a heterogeneous group of glycated proteins. The tetrazolium dye reaction is not fully characterized; therefore test results should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

The performance characteristics of this test have not been established for use to screen for the presence of diabetes mellitus.

Severe hypoalbuminemia (serum albumin concentrations lower than 30-35 g/L), usually found in conditions such as nephrotic syndrome, may lower serum fructosamine concentrations and not reflect the true degree of glycemia (5, 9).

It has been reported that uremic or thyrotoxic states with characteristic accelerated protein catabolism may slightly decrease fructosamine concentrations even with normal or near-normal albumin concentrations. It is also reported that to a lesser degree hypothyroid patients may show a slight increase in fructosamine concentrations (5, 10)

In general, a serum fructosamine concentration uncorrected for protein concentration accurately depicts the level of glycemia provided that there is not coexisting severe hypoproteinemia (5).

## REAGENT PERFORMANCE

1. Linearity: The assay is linear in the range from 1.3 mmol/L to 8.5 mmol/L fructosamine. Serum samples with fructosamine concentrations greater than 8.5 mmol/L should be diluted with physiological saline (sodium chloride: 150 mmol/L in water) and reassayed. Correct the final value by multiplying by the dilution factor.
2. Correlation: Assay results obtained with this reagent from 92 serum samples ranging in fructosamine concentrations from 1.51 mmol/L to 8.28 mmol/L were compared to the fructosamine reagent from Isolab, Inc. The correlation coefficient was 0.999 and the regression equation was y = 1.01x + 0.06.
3. Precision:

Within Run

| | Low | Medium | Medium High | High |
|---|---|---|---|---|
| Mean (mmol/L) | 1.93 | 3.06 | 3.99 | 7.70 |
| S.D. | 0.018 | 0.02 | 0.02 | 0.04 |
| C.V.% | 0.9 | 0.7 | 0.5 | 0.6 |
| N | 21 | 19 | 21 | 22 |

Run to Run

| | Low | Medium | Medium High | High |
|---|---|---|---|---|
| Mean (mmol/L) | 1.94 | 3.07 | 4.01 | 7.64 |
| S.D. | 0.02 | 0.05 | 0.04 | 0.08 |
| C.V.(%) | 1.0 | 1.6 | 1.0 | 1.0 |
| N | 51 | 48 | 51 | 54 |

## REFERENCE RANGE

The reference values for non-diabetics and diabetics are reported from literature as indicated in the bibliography.

1. Non-diabetic values.

The following concentrations of fructosamine (mmol/L) were from apparently normal, healthy individuals with no history or indication of diabetes.

| Population | N | Mean (mmol/L) | SD | Reference Range* | Observed Range | Reference No. |
|---|---|---|---|---|---|---|
| Males | 96 | 2.20 | 0.31 | 1.59-2.81 | 1.50-3.17 | 11 |
| Females | 98 | 2.25 | 0.36 | 1.89-2.61 | 1.67-3.84 | 11 |
| Males | 97 | 2.21 | 0.43 | 1.37-3.05 | 1.29-4.0 | 12 |
| Females | 86 | 2.13 | 0.32 | 1.51 -2.75 | 1.55-3.22 | 12 |

*Reference Range = the mean ± 1.96 SD

2. Known diabetic values.

The following concentrations of fructosamine (mmol/L) were from serum samples from known diabetics.

| Population | N | Mean (mmol/L) | SD | Observed Range | Reference No. |
|---|---|---|---|---|---|
| New/uncontrolled diabetics | 89 | 3.28 | 0.68 | 2.05-5.32 | 11 |
| Known diabetics under treatment | 57 | 2.54 | 0.73 | 1.52-4.63 | 12 |

It is recommended that each laboratory establish its own reference range.

## REFERENCES


1. Mosca, A., et al., *Clin Chem.* 33 (7): 1141-1146, 1987.
2. Armbruster, D.A., *Clin Chem.* 33: 2153-2163, 1987.
3. Bunn, H.F., et al., *Science* 200: 21-27, 1978.
4. Johnson, R.N., et al., *Clin Chem. Acta* 127: 87-95, 1982.
5. Day, J.F., et al., *Diabetes* 29: 524-527, 1980.
6. Baker, J.R. et al., *Brit Med J.* 287: 863-867, 1983.
7. Lim, Y.S., and Stanley, M.J., *Clin Chem.* 31 (5): 731, 1985.
8. Roberts, A.B., and Baker, J.R., *American Journal Obst. Gyn*. 154, No. 5,1 027-1 030, 1989.
9. Lloyd, D. and Marples, J., *Clin Chem*. 32 (10): 1986.
10. Lloyd, D. and Marples, J., *Clin Chem*. 30 (10): 1686-1688, 1984.
11. Teupe, B. (manuscript in preparation).
12. Rosenthal, M., and Olenich, J.L., *Clin Chem*. 34 (2): 360-363, 1988.


IVD **For in vitro diagnostic use**

**See package insert for proper use**


**CLINIQA CORPORATION**
288 Distribution St.
San Marcos, CA 92078
USA

**SALES AND TECHNICAL SUPPORT**
P: 800 728 5205
+1 760 744 1900
F: +1 760 571 5198
www.cliniqa.com

**FOR ORDERS AND CUSTOMER SERVICE**
P: 800 728 5205
+1 760 744 1900
F: +1 760 571 5197
csgroup@cliniqa.com

EC REP
CEpartner4U
Esdoornlaan 13
3951 DB Maarn, The Netherlands
P: +31 (0)6 516 536 26


## RE-ORDER INFORMATION
## Fructosamine Reagent

REF **Catalog No.**
R85317

Made in the USA

32937_01 11/24/09