K-*ASSAY*®

Fructosamine + Calibrator

For the Quantitative Determination of Fructosamine

Cat. No. KAI-043 / KAI-050 / KAI-048C

INTENDED USE

For the quantitative determination of Fructosamine in human serum. FOR *IN VITRO* DIAGNOSTIC USE ONLY.

CLINICAL SIGNIFICANCE

The determination of fructosamine is most commonly performed for the evaluation of glycemic control in diabetes. Fructosamine values provide an indication of glucose levels over the preceding 2-3 weeks. A higher fructosamine value indicates poorer glycemic control.[1,2]

INTRODUCTION AND SUMMARY

Glycated proteins are formed by a non-enzymatic reaction between glucose and protein in which unstable Schiff bases are formed, followed by an Amadori conversion to form stable ketoamines.[3] These glycated proteins include glycohemoglobin, glycoalbumin and glycated total protein. Fructosamine is a term that has come into acceptance and refers to both glycoalbumin and glycated total protein.[4] As the average life span of these proteins is about 2-3 weeks, the level of fructosamine provides a reflection of the average glucose concentration over that time.[5]

Fructosamine and glycohemoglobin are both used to monitor diabetic control. However, each assay provides information for a specific time frame that is related to the analyte being measured. Since the life span of hemoglobin is closer to 6-8 weeks, glycohemoglobin measurements reflect the average glucose concentration over this longer period of time.[5] Therefore, in comparison to glycohemoglobin determinations, fructosamine provides an index of intermediate-term diabetic control as opposed to the longer term for glycohemoglobin. Also, because of the shorter life span of the glycated albumin and total proteins, fructosamine measurements are more sensitive to changes in diabetic control. This provides a means to alert the physician to improvement, or deterioration in control much earlier than glycohemoglobin determinations.[6]

There have been several methods developed for the determination of fructosamine. These methods include phenylhydrazine, furosine, affinity chromatography and several colorimetric procedures.[7] A procedure using furosine and HPLC is accepted as the reference method however, a colorimetric procedure using nitroblue tetrazolium (NBT) has gained popularity due to its speed, reproducibility and ease of automation.[8] The reagent presented here is a modification of the commonly used NBT method.

PRINCIPLE OF TEST

The fructosamine reagent set is based on the ability of ketoamines to reduce NBT to a formazan dye under alkaline conditions. The rate of formazan formation, measured at 550 nm, is directly proportional to the fructosamine concentration.

KIT COMPOSITION

KAI-043 / KAI-050:

Fructosamine Buffer: Carbonate buffer 100 mM, pH 10.35 ± 0.1, sodium azide less than 0.1%.

Fructosamine Substrate: Nitroblue tetrazolium (NBT) 0.57 mM, surfactant, non-reactive stabilizers and fillers.

Fructosamine Calibrator: pooled human serum containing buffers, stabilizers and fillers.

KAI-048C :

Fructosamine Calibrator: pooled human serum containing buffers, stabilizers and fillers.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Rx only.
2. Do not use reagents past their expiration date stated on each reagent container label.
3. Do not pipette by mouth. Avoid ingestion and contact with skin.
4. Reagents in this kit contain sodium azide (less than 0.1%) as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.
5. All specimens, calibrators and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)[9]
6. Human serum was used in the manufacture of the calibrator. Each donor unit was tested and found negative, or non-reactive for HbsAb, HCV and HIV.

REAGENT PREPARATION

Reconstitute the Fructosamine Substrate with the amount of Fructosamine Buffer specified on the vial label. Swirl gently to dissolve. The Fructosamine Calibrator is supplied as a liquid stable serum based product. It is ready to use upon opening.

REAGENT STORAGE AND STABILITY

1. Un-reconstituted reagents are stable until the expiration date on the kit label when stored at 2-8°C.
2. Upon reconstitution, the substrate should be stored at 2-8°C for best results.
3. Reconstituted substrate is stable for 7 days if stored at room temperature (15-25°C) or 30 days if stored refrigerated (2-8°C).
4. After opening, calibrator is stable for 30 days stored at 2-8°C.

SPECIMEN COLLECTION AND STORAGE

1. Human serum, separated from the cells as quickly as possible, is the specimen of choice.
2. Collect specimens per NCCLS document H4-A3.[10]
3. Avoid hemolysis or contamination of the sample with hemoglobin as glycated hemoglobin will react in the same manner as fructosamine.
4. Serum specimens are stable for one week if stored at 2 to 8°C. Storage at -20°C is not recommended.[11]

PROCEDURE

Materials Supplied

<u>KAI-043 Fructosamine + Calibrator</u>	
Reagent 1 (R1) Buffer	1 x 120 mL
Reagent 2 (R2) Substrate	5 x 11 mL
Fructosamine Calibrator	1 x 2 mL
<u>KAI-050 Fructosamine + Calibrator (L)</u>	
Reagent 1 (R1) Buffer	1 x 120 mL
Reagent 2 (R2) Substrate	10 x 11 mL
Fructosamine Calibrator	1 x 2 mL
<u>KAI-048C Fructosamine Calibrator</u>	
Fructosamine Calibrator	1 x 2 mL

Materials Required But Not Supplied

1. Pipetting devices
2. Test tubes/rack
3. Timing device
4. Heating block
5. Spectrophotometer capable of reading at 550 nm.
6. Fructosamine Controls, cat. no. K52C-6M.

Assay Procedure (Automated-General)

Wavelength:	550 nm
Assay Type:	Kinetic with Calibrator
Sample/Reagent Ratio:	1:21
Reaction Direction:	Increasing
Temperature:	37°C
Lag Time:	600 seconds
Read Time:	300 seconds
Reference Range:	1.61-2.68 mmol/L

Assay Procedure (Manual Method)

1. Label tubes “Calibrator”, “Control”, “Sample”, etc.
2. Pipette 1.0 mL substrate into all tubes and prewarm at 37°C for five minutes.
3. Add 0.05 mL (50 µL) of specimen to each respective tube at timed intervals.
4. After exactly ten (10) minutes at 37°C read the absorbance of each tube at 550 nm (A_1). Return each tube to 37°C.
5. After exactly five (5) more minutes at 37°C, read tubes again at 550 nm (A_2).
6. To determine results see “Calculations”.

CALIBRATION

This assay requires the use of a fructosamine calibrator. The calibrator included in the kit is recommended. The use of a fructosamine calibrator from another source may produce inaccurate results. The procedure should be calibrated according to the instrument manufacturer’s calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

QUALITY CONTROL

Serum controls with known normal and abnormal fructosamine values should be run routinely to monitor the validity of the reaction. These controls should be run at least with every working shift in which fructosamine determinations are performed. It is strongly recommended that each laboratory establish its own frequency of control determination.

CALCULATIONS

A = Absorbance

$$\frac{A_2\ \text{Sample} - A_1\ \text{Sample}}{A_2\ \text{Calibrator} - A_1\ \text{Calibrator}} \times \text{Conc. of Calibrator} = \text{Fructosamine in Sample}$$

Example: if A_1 Sample = 0.100 and A_2 Sample = 0.600,
A_1 Calibrator = 0.100 and A_2 Calibrator = 0.400,
And Concentration of Calibrator = 3.0 mmol/L then:

$$\frac{0.600 - 0.100}{0.400 - 0.100} \times 3.0\ \text{mmol/L} = 5.0\ \text{mmol/L}$$

LIMITATIONS OF PROCEDURE

1. The procedure described is linear to 10.0 mmol/L. Samples with values exceeding 10.0 mmol/L should be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
2. Hemoglobin greater than 200 mg/dL may give falsely elevated results.

PERFORMANCE

Sensitivity: An investigation of the absorbance change per minute for ten replicates of two samples, with known concentrations of fructosamine, indicated that an absorbance change per minute of 0.042 was approximately equivalent to 1 mmol/L fructosamine.

Precision: Precision studies were performed following a modification of the procedure contained in NCCLS document EP5-T2.[15]

Within Day (n=20)			Day to Day (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
1.97	0.04	2.0	1.91	0.06	3.1
5.57	0.10	1.8	5.72	0.14	2.4

Correlation: Results obtained with this reagent (y), in 45 samples ranging in fructosamine from 1.17 – 5.94 mmol/L, were compared with those obtained in the same samples using a reagent (x) based on the same methodology. The correlation coefficient was 0.988 and the regression equation was y = 0.88x + 0.28 (Std Err of Y Est = 0.19).

Assay Range: 1.0 – 10.0 mmol/L

INTERFERENCE

1. All interference studies were performed according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry.[12]
2. Bilirubin to 20 mg/dL has been demonstrated to have a negligible effect (<5%) on fructosamine results using this method.
3. Hemoglobin to 200 mg/dL has been demonstrated to have a negligible effect (<5%) on fructosamine results using this method.
4. Glucose to 600 mg/dL has been demonstrated to have a negligible effect on fructosamine results using this method.
5. See Young, et al. for other interfering substances.[13]

EXPECTED VALUES

1.61 – 2.68 mmol/L[14]
It is strongly recommended that each laboratory establish its own normal range.

REFERENCES

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11. Burtis, C.A., Ashwood, E.R., Tietz Textbook of Clinical Chemistry, Philadelphia (PA), W.B. Saunders, p. 797 (1999).
12. NCCLS, "National Evaluation Protocols for Interference Testing", Evaluation Protocol Number 7, Vo. 4, No. 8, June 1984.
13. Young, D.S., et al, Clin. Chem. 21:1D (1975).
14. Burtis, C.A., Ashwood, E.R. Tietz Fundamentals of Clinical Chemistry, Philadelphia (PA), W.B. Saunders, p. 791 (1996).
15. NCCLS document, "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
STD	Calibrator
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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