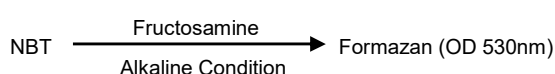


Fructosamine Assay Kit (Colorimetric) (#BN00692)

(Catalog # BN00692; 100 assays; Store at -20°C)

I. Introduction:

Fructosamines are stable glycosylated proteins that are formed by a non-enzymatic reaction between glucose and serum proteins (usually albumin). Elevated concentrations of fructosamine can be found in serum samples of diabetic patients and its detection can be used to assess the glycemic status of diabetics. The half-life of albumin is shorter when compared to hemoglobin ($t_{1/2}$ = 20 days vs. 50 days respectively). Thus, fructosamine levels reflect the efficacy of treatment in diabetic patients during short-term periods, and provide earlier and more sensitive detection for diabetes than many other carbohydrate tests. Assay Genie's Fructosamine Assay Kit is a microplate-based colorimetric assay for the direct determination of fructosamine levels in serum. The assay is based on the ability of fructosamine to reduce nitroblue tetrazolium (NBT), forming a colored end-product (purple) under alkaline conditions. The formation rate of formazan is proportional to the concentration of fructosamine in samples and the increase in absorbance (OD 530nm) can be monitored using a spectrophotometer. The kit includes a Thiol Blocking Reagent and a Sample Cleaning Mix that minimizes the interference of other endogenous reducing agents which ensures accurate measurements of Fructosamine in biological samples. The assay is simple, reproducible and can detect as low as 20 μ mol/L of fructosamine in samples.



II. Applications:

- Measurement of Fructosamine levels in serum

III. Sample Type:

- Serum

IV. Kit Contents:

Components	BN00692	Cap Code	Part Number
Fructosamine Buffer A	25 ml	WM	BN00692-1
NBT (in DMF)	580 μ l	Red	BN00692-2
Thiol Blocking Reagent	1 vial	Amber	BN00692-3
Sample Cleaning Mix (Lyophilized)	1 vial	Green	BN00692-4
Fructosamine Buffer B	40 ml	NM	BN00692-5
Fructosamine Calibrator (3.2 mM)	180 μ l	Yellow	BN00692-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Fructosamine Buffers A and B:** Store at 4 °C. Bring to room temperature before use.
- **NBT:** Aliquot and Store at -20°C. Protect from light. Bring to room temperature before use.
- **Thiol Blocking Reagent:** Reconstitute with **400 μ l Fructosamine Buffer A**. Vortex and Mix Well. Store at -20°C. When dissolved, use within 2 month.
- **Sample Cleaning Mix:** Reconstitute with **1 ml Fructosamine Buffer A**. Allow contents to dissolve intensively. Aliquot and keep at -20 °C. When dissolved, use within 2 month.
- **Fructosamine Calibrator:** Upon received, **store at 4°C**. Stable for 2 months at 4 °C after opening.

VII. Fructosamine Assay Protocol:

- Sample and Fructosamine Calibrator Preparation:** Add 10 μ l of undiluted samples or 10 μ l of dH₂O into wells of a clear 96-well plate and label as "Sample" and "Background". For positive control, add 3 μ l of Fructosamine Calibrator into well(s), adjust the volume to 10 μ l with Fructosamine Buffer A and label as "Fructosamine Calibrator(s)".

Note:

3 μ l of Fructosamine Calibrator is equivalent to 9.6 nmol Fructosamine (see step 6).

- Thiol Blocking Reagent and Sample Cleaning Mix Preparation:** Prepare Reagent Mix by adding 2 μ l of Reconstituted Thiol Blocking Reagent, 5 μ l of Reconstituted Sample Cleaning Mix with 30 μ l Fructosamine Buffer A (total volume is 37 μ l/per well). Prepare enough reagents for the number of samples to be assayed. Add Prepared Reagent Mix (37 μ l) to all wells containing "Sample", "Background" and "Fructosamine Calibrator". Mix well.

Note:

The test is very sensitive to temperature; pre-warm Fructosamine Buffer A to 37 °C before adding to well(s).

- NBT Preparation:** Add 3 μ l NBT to each wells containing "Sample", "Background" and "Fructosamine Calibrator". *Partial volume of each well should be 50 μ l. Mix well. Pre-Incubate the plate at 37 °C for 10 min to remove interferences*, avoid light.

4. Fructosamine Reaction: Add 200 μ l of **Fructosamine Buffer B** to each well containing the “Sample”, “Background”, “Fructosamine Calibrator”. Mix well. *Total volume in every well should be 250 μ l.* **Incubate the plate at 37°C for 5 min**, avoid light.

Notes:

1) *The test is very sensitive to temperature*; pre-warm Fructosamine Buffer B to 37 °C before adding to well(s).

2) We suggest use a multichannel pipette to assay multiple samples/wells.

5. Measurement: After 5 min. incubation, measure absorbance 530 nm at 37°C at **two specific time points** ($t_1=5$ min, OD₁ and $t_2=15$ min, OD₂).

Note:

The first 5 minutes of incubation (after the addition of Fructosamine Assay Buffer B) minimizes the effect of non-specific reducing substances for calculating Fructosamine levels. Do not use the first 5 min OD 530 nm readings for calculating fructosamine concentrations.

6. Calculation: Calculate the change in absorbance during the 10 min interval ($A=OD_2-OD_1$) of each wells labeled as Sample, Calibrator and Background. Subtract “Background” readings from “Sample” and from “Calibrator”, respectively.

$$\text{Fructosamine amounts in sample} = \frac{[A(\text{Sample}) - A(\text{Background})]}{[A(\text{Calibrator}) - A(\text{Background})]} * 9.6 = B \text{ (nmol)}$$

$$\text{Fructosamine concentration in sample} = B/V \text{ (nmol/ml, } \mu\text{M)}$$

Where: **B** = Fructosamine amount (nmol)
V = the sample volume added into reaction well (ml)
9.6 = Fructosamine amounts (nmol) of 3 μ l of Fructosamine Calibrator

Sample Fructosamine concentration can be expressed in μ mol/L (μ M).

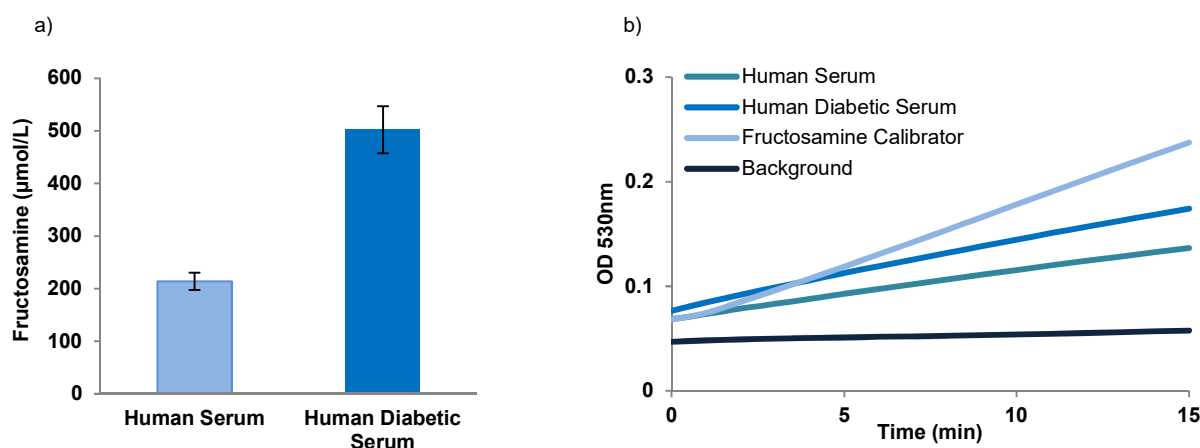


Figure: (a) Fructosamine Reaction Curves. (b) Fructosamine concentrations (μ mol/L): Estimated Fructosamine concentrations are 222.4 \pm 20.7 μ mol/L in healthy human Serum (10 μ l) and 458.8 \pm 43.9 μ mol/L in Human Diabetic Serum (10 μ l), respectively. Assays were performed following the kit protocol.