Fructosamine Assay Kit

ARG83423 Fructosamine Assay Kit can be used to measure Fructosamine in serum, plasma and other biological fluids.

Catalog number: ARG83423

Package: 96 wells

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INTRODUCTION

Fructosamine, which is a measure of non-enzymatic glycation of circulating proteins including albumin, globulins, and lipoproteins, has evolved to be a reasonable alternative to HbA1c measurement in situations where HbA1c is not reliable. Because albumin is the most abundant of the serum proteins, fructosamine is predominantly a measure of glycated albumin (GA), which represents the percent of albumin that is glycated. Fructosamine and GA have a potential role in the diagnosis, monitoring, and management of diabetes.

PRINCIPLE OF THE ASSAY

This Fructosamine Assay Kit is a simple colorimetric assay that measures the amount of Fructosamine present in serum, plasma and other biological fluids. 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 can be measured at a colorimetric readout at 540 nm. The concentration of Fructosamine in the samples is then determined by comparing the O.D. 540 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well microplate	1 plate	
Standards	1 vial (lyophilized)	4°C
Reaction Buffer	8 mL	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Assay Buffer	2 x 30 mL	4°C
Diluent	5 mL	4°C
Stop Solution	5 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 540 nm
- Centrifuge
- Deionized or Distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- All reagents should be mixed by gentle inversion or swirling prior to use.

Do not induce foaming.

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note: For liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Reagent Dye:** Reconstitute the Reagent Dye with **5 ml** of **Diluent**. Allow the Reagent Dye keep on bench for few minutes. Make sure the Reagent Dye is dissolved completely and mixed thoroughly before use.
- **Standards:** Reconstitute the Standards with **1 ml Assay Buffer** to yield 4 µmol/mL Standard stock. Allow the Standards stock keep on bench for few minutes. Make sure the Standards stock is dissolved completely and mixed thoroughly before use. Add **0.5 ml Standard stock** into **0.5 ml Assay Buffer**, the concentration will be **2 µmol/mL**. Perform 2-fold serial dilutions of the top standards to make the standard curve.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **20 µl** of **Standard** into Standard wells.
2. Add **20 µl** of **Sample** into Sample wells.
3. Add **80 µl** of **Reaction Buffer** into all wells.
4. Mix well. Incubate at **37°C** for **10 min**.
5. Add **50 µl** of **Reagent Dye** into all wells.
6. Mix well. Incubate at **37°C** for **15 min**.
7. Add **50 µl** of **Stop Solution** into all wells.
8. Read the OD at **540 nm**.

Summary of Glucoamylase Assay Procedure

Reagent	Standard	Sample	Blank
Standard	20 µl	-	-
Sample	-	20 µl	-
Distilled water	-	-	20 µl
Reaction Buffer	80 µl	80 µl	80 µl
Mix well. Incubate at 37°C for 10 min			
Reagent Dye	50 µl	50 µl	50 µl
Mix well. Incubate at 37°C for 15 min			
Stop Solution	50 µl	50 µl	50 µl
Read the OD at 540 nm.			

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Calculation:

   A. Definition:

   $C_{Standard}$: the standard concentration, 2 µmol /ml;

   V_{Sample}: the volume of reaction sample, 20 µl = 0.02 ml;

   $V_{standard}$: the volume of standard, 20 µl = 0.02 ml;

   B. Formula:

   a). According to the volume of sample

   Fructosamine ((µmol/ml) =

   $[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

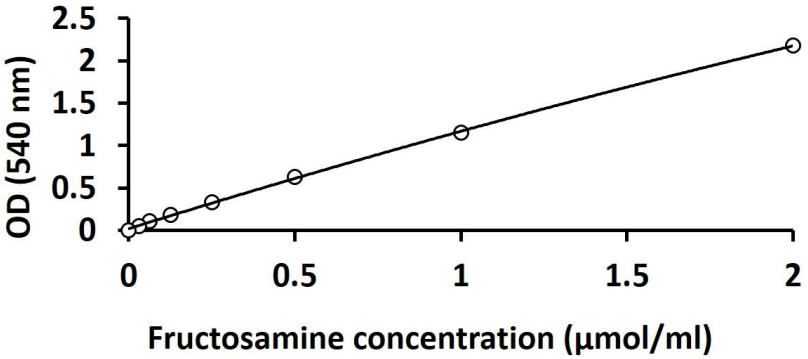
   $=2 \times (OD_{Sample} - OD_{blank}) / (OD_{Standard} - OD_{Blank})$

5. Detection range:

   The detection range is from 0.01 µmol/ml- 2 µmol/ml.
6. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Fructosamine Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.01 µmol/mL