Fructosamine

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1243

(Version 1.3A)

Detection and Quantification ofFructosamineContentin Serum,

Plasma and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Fructosamines are stable glycated 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 (t1/2= 20 days vs. 50 days respectively). Thus, fructosamine levels reflect the efficacy of treatment in diabetic patients during short-term periods, and provide earlierand more sensitive detection for diabetes than many other carbohydrate tests.

FructosamineColorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of Fructosamine in a variety of samples. 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, is proportional to the fructosamine concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 2	4 °C
Diluent	5 ml x 1	4 °C
Reaction Buffer	8 ml x 1	4 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Stop Solution	5 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Convection oven
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 mlAssay Bufferto generate 4 mmol/L of standardstock solution, store at -20 °C for 1 monthafter reconstitution.Then dilute to2 mmol/Lstandard top solutionby adding0.5 ml stock solution into 0.5 ml Assay Buffer**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be2/1/0.5/0.25/0.125/0.0625/0.0312/0.0156mmol/L.

Dye Reagent: Add 5 ml Diluent to dissolve before use. Keep in dark and store at 4 C for 2 weeks or -20 °C for 3 monthsafter reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For serum or plasma samples

Detect directly, or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	80µl	80µl	80µl
Sample	20µl	--	--
Standard	--	20µl	--
Distilled water	--	--	20µl
Mix, incubate at 37 °C for 10minutes.			
Dye Reagent	50 µl	50 µl	50 µl
Mix, incubate at 37 °C for 15minutes.			
Stop Solution	50 µl	50 µl	50 µl
Mix, record absorbance measured at 540 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

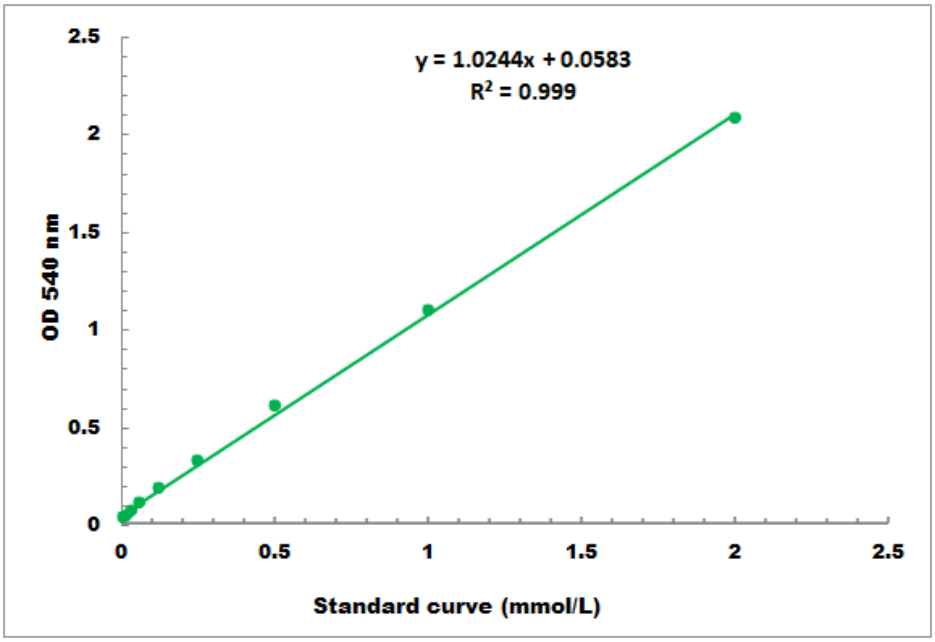
$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

VII. CALCULATION

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 2 mmol/L