Fructosamine

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1243

(Version 1.2A)

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
Stop Solution	5 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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Note:

Dye Reagent: add 5 ml Diluent to dissolve before use.

Standard: add 1 ml Assay Buffer to dissolve before use;then add 0.5 ml standard solution into 0.5 ml Assay Buffer, mix, the concentration will be 2 mmol/L.

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. SAMPLE PREPARATION

1.For serum or plasma samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	20µl	--	--
Standard	--	20µl	--
Distilled water	--	--	20µl
Reaction Buffer	80µl	80µl	80µl
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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1.According to the volume of serum or plasma

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

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

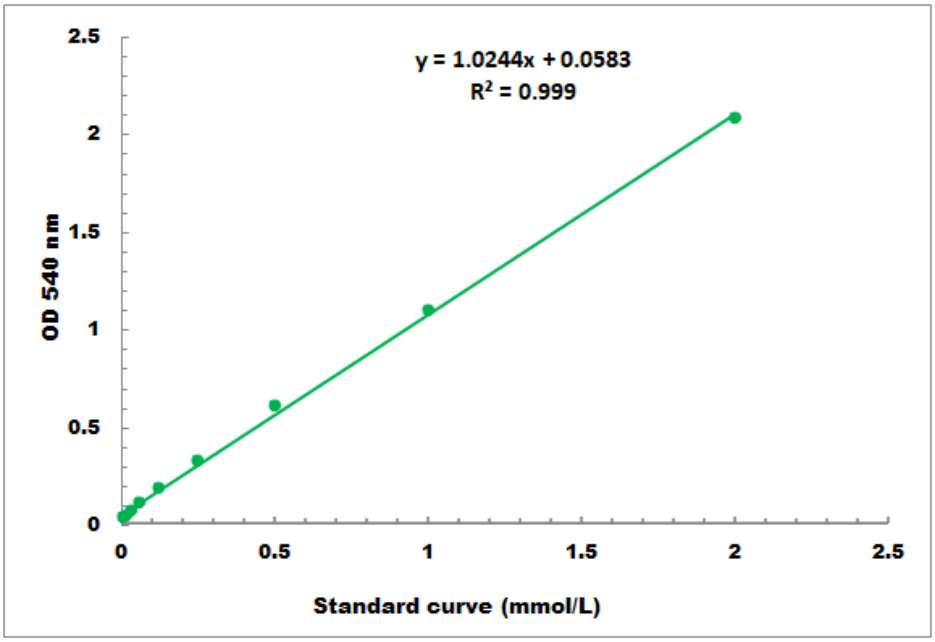
$C_{Standard}$: the concentration of standard, 2 mmol/L = 2μmol/ml;

$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml.

VII. TYPICAL DATA

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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES