

Fructosamine Colorimetric Microplate Assay Kit

Cat #: orb707398 (manual)

For Research Use Only. Not For Use in Diagnostic Procedures!

Detection and Quantification of Fructosamine Content in Serum, Plasma and Other biological fluids Samples.

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.

Fructosamine Colorimetric 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 nitro blue 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.

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 | |

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

REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Assay Buffer to generate 4 mmol/L of standard stock solution, store at -20 °C for 1 month after reconstitution. Then dilute to 2 mmol/L standard top solution by adding 0.5 ml stock solution into 0.5 ml Assay Buffer. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312/0.0156 mmol/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 months after reconstitution.

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

SAMPLE PREPARATION

1. For serum or plasma samples

Detect directly or dilute with Assay Buffer.

ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent* | Sample** | Standard | Blank |
|--|----------|----------|-------|
| Reaction Buffer | 80µl | 80µl | 80µl |
| Sample | 20µl | -- | -- |
| Standard | -- | 20µl | -- |
| Distilled water | -- | -- | 20µl |
| Mix, incubate at 37 °C for 10 minutes. | | | |
| Dye Reagent | 50 µl | 50 µl | 50 µl |
| Mix, incubate at 37 °C for 15 minutes. | | | |
| 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.

CALCULATION

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

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

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

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

Slope: the absorbance slope of standard curve

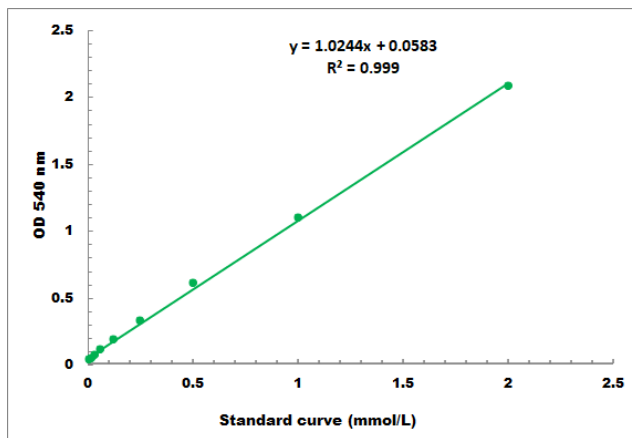
C_{Standard}: the standard concentration, mmol/L = μmol/mL

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

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

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