Fructosamine Assay Kit

Catalog No.: abx298916

Size: 100 tests

Storage: Store the Dye Reagent and Standard at -20°C, and all other components at 4°C.

Application: For quantitative detection of Fructosamine concentrations in serum, plasma, and other biological fluids.

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

Introduction: Fructosamine (FMN) is a substance formed by proteins in the non-enzymatic saccharification of glucose. FMN concentration is positively correlated with blood sugar level and is relatively stable. Its measurement is not affected by blood sugar. Since the lifetime of plasma proteins is 17 to 20 days, fructosamine can reflect the average blood glucose level over a period of 1 to 3 weeks. It provides a shorter-term solution for monitoring average blood glucose levels over shorter periods as opposed to measuring glycosylated hemoglobin which reflects a period of approximately 3 months.

Abbexa's Fructosamine Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Fructosamine concentrations. Fructosamine reduces nitro blue tetrazolium (NBT) to form a purple-colored reaction product, with an absorption maximum at 540 nm. The intensity of the color is proportional to the concentration of Fructosamine, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 2 × 30 ml
3. Reaction Buffer: 8 ml
4. Dye Reagent: 1 vial
5. Dye Reagent Diluent: 5 ml
6. Stop Solution: 5 ml
7. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (540 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**
  Add 5 ml of Dye Reagent Diluent into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.
- **Standard Solution**
  Add 1 ml of Assay Buffer into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 0.5 ml of this solution and add 0.5 ml of Assay Buffer to prepare the Standard Solution (concentration 2 mmol/L).

2. Sample

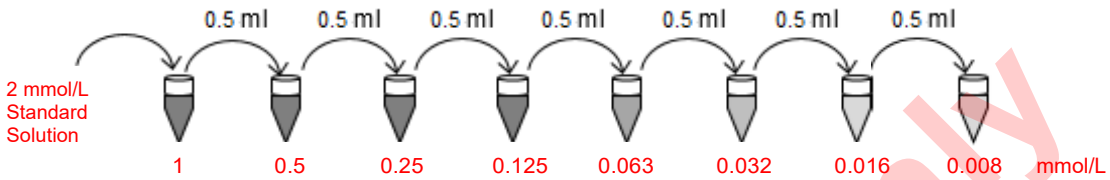
- **Liquid samples**
  Liquid samples such as serum and plasma can be used directly or diluted with Assay Buffer.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 9 tubes with 2 mmol/L, 1 mmol/L, 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.063 mmol/L, 0.032 mmol/L, 0.016 mmol/L and 0.008 mmol/L. Aliquot 0.5 ml of Assay Buffer into each tube. Add 0.5 ml of 2 mmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of prepared standards (2 mmol/L, 1 mmol/L, 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.063 mmol/L, 0.032 mmol/L, 0.016 mmol/L and 0.008 mmol/L) to the standard wells.
5. Add 20 µl of distilled water to the blank wells.
6. Add 80 µl of Reaction Buffer to all wells.
7. Tap the plate gently to mix. Incubate at 37 °C for 10 minutes.
8. Add 50 µl of Dye Reagent Solution to all wells.
9. Tap the plate gently to mix. Incubate at 37 °C for 15 minutes.
10. Add 50 µl of Stop Solution to all wells.
11. Tap the plate gently to mix. Read and record absorbance at 540 nm.

C. Calculations

Fructosamine content per ml serum or plasma:

$$\mathbf{Fructosamine\ (\mu mol/ml)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{2} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Standard}}$	Concentration of highest standard (2 mmol/L= 2 µmol/ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.02 ml)
$\mathbf{OD_{Sample}}$	The OD value of the sample
$\mathbf{OD_{Blank}}$	The OD value of the blank
$\mathbf{OD_{Standard}}$	The OD value of the highest standard