CREATIVE BIOMART®
Assay Kit

Fructosamine Enzymatic Assay Kit

Product Information

Cat

Kit-2413

Cat.No. Kit-2413

Product Overview

Fructosamine Enzymatic Assay Kit is a plate-based colorimetric assay for the determination of Fructosamine in serum samples. The kit uses a spectrophotometric assay to detect Fructosamine directly from serum samples, enabling researchers to detect Fructosamine levels in animal serum and other tissue matrices.

Description

Fructosamines are stable carbohydrate-protein complexes that form in the blood by glycation reactions between sugars and primary amines. In diabetes, blood sugars are measured by either monitoring current blood glucose level or by glycated hemoglobin (HbA1c). Similar to the HbA1c test, fructosamine testing calculates the fraction of total serum proteins that have undergone glycation and typically reflect albumin glycation. Since the half-life of albumin is approximately 20 days, the fructosamine level reflects 1-2 week changes in blood glucose. Therefore, fructosamine testing provides earlier and more sensitive detection for diabetes than many other carbohydrate tests.

Fructosamine Enzymatic Assay Kit is a simple, direct and automationcompatible method for measuring fructosamine levels in serum. This kit is based on the ability of ketoamines to directly reduce nitroblue tetrazolium (NBT) to colored formazan dye under alkaline conditions. The absorbance measured at 550 nm, is proportional to the concentration of fructosamine in the sample. The kit also comes with a control solution containing a fructosamine standard which can be used to calibrate the assay

Stability

The shelf life is 6 months after receipt when the kit is properly stored. After opening, the Fructosamine Standard is stable for 30 days stored at 4°C. Reconstituted Fructosamine Reagent is stable for 30 days if stored refrigerated at 4°C.

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Storage

Store the kit at 4 °C.

Warning

1. Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve.
2. The assay is not influenced by hemoglobin values up to 200mg/dl or by bilirubin levels up to 20mg/dl or by glucose up to 600 mg/dl.
3. Interference from grossly hemolyzed specimens is correctable by use of a serum/plasma blank.

Size

96 wells

Kit Components

Microtiter Plate, One, 4°C – 25 °C
Fructosamine Reagent, Bottle, 4 °C
Reagent Buffer, 2 x 20 mL, 4 °C
Fructosamine Standard (3.2 mmol/L), 0.6 mL, 4 °C
Standard Dilution Buffer, 1.5 mL, 4 °C

Materials Required but Not Supplied

Microtiter plate reader (550 nm).
Heating block or temperature incubator.
Timer.
Centrifuge to prepare serum samples.
Deionized or distilled water.
PBS (phosphate buffer saline, pH 7.3)
1.5 mL microfuge tubes.
Multichannel pipet or repeating pipettor (recommended but not required).

Features & Benefits

1. High sensitivity and large assay range (1 - 8 mmol/L).

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2. A rapid (15 minute) and robust enzyme-based assay which does not require expensive instrumentation.
3. High reproducibility.

Preparation

1. Carefully prepare at least 30 µL of serum using standard production procedure (if determinations are performed in singlet then 15 µL is sufficient). Avoid hemolysis or contamination of the sample with hemoglobin as glycated hemoglobin will react in the same manner as fructosamine.
2. Serum samples are stable for one week if stored at 4 °C.
Note: Samples with fructosamine values above 8 mmol/L should be diluted 1:1 with PBS and re-tested. Multiply results by two.

Assay Protocol

Set up:
The test is very sensitive to temperature; pre-warm all reagents to 37 °C. Turn on the plate reader and allow lamp to warm up. Set temperature of plate reader to 37 °C before reading. Adjust the wavelength of the plate reader to 550 nm.

Reagent Preparation:

Preparation of Reagent:
Mix To reconstitute the Fructosamine Reagent, add exactly 30 mL of Reagent Buffer to the Fructosamine Reagent bottle. Mix by swirling or inverting the bottle 10 - 12 times. Allow contents to dissolve for 10 minutes at room temperature.
IMPORTANT: The reconstituted Reagent Mix can be left at room temperature for short periods (30-60 minutes) prior to use. Between uses, the reconstituted Fructosamine Reagent should be stored at 4°C (for up to 30 days). Discard the reconstituted Reagent thirty days after reconstitution.
Prewarm the reconstituted Reagent Mix to 37°C before performing the assay.

Preparation of Fructosamine Control Dilutions for Standard Curve:
1. Label 6 microfuge tubes: 1, 2, 3, 4, 5, 6.

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2. Dilute the Fructosamine Standard using the Standard Dilution Buffer as described in the following table. After dilution, briefly mix each tube.

Tube#	Volume Fructosamine Standard (3.2 mmol/L)	Volume Standard Dilution Buffer	Fructosamine Concentration (mmol/L)
1	60 µl	0 µl	3.20
2	50 µl	10 µl	2.67
3	40 µl	20 µl	2.13
4	30 µl	30 µl	1.60
5	20 µl	40 µl	1.07
6 (Neg)	0 µl	60 µl	0

Sample Test Procedure

1. Add 300 µL of prewarmed Fructosamine Reagent to the wells and prewarm the microplate in the incubator at 37°C for 15 minutes.
2. Add 15 µL of each sample or standard (in duplicate) to the microplate wells. Mix briefly.
3. Measure the absorbance immediately of each sample at 550 nm (= A1). Incubate the microplate at 37°C.
4. After exactly 15 minutes at 37°C, measure the absorbance of each sample again at 550 nm (= A2).
5. For each sample or standard, subtract the initial absorbance (A1) from the absorbance at the 15 minute time point (A2) to obtain the change in absorbance (= A2 - A1).

Analysis

There is a linear relationship between the concentration of fructosamine in the sample and the change in absorbance during the 15 minute interval (= A2 - A1). Therefore, a standard curve used to calculate the fructosamine concentration in sera samples can be constructed by plotting the mean corrected absorbance values difference (A2-A1) for each of the diluted fructosamine standards as a function of the fructosamine concentration. The concentration of the fructosamine in serum samples can be determined using this standard curve.