CREATIVE BIOMART®
Assay Kit

Fructosamine Assay Kit (Colorimetric)

Product Information

Common Name

Fructosamine

Cat.No. Kit-1020

Product Overview

Fructosamine Assay Kit is a microplate-based colorimetric assay for the direct determination of fructosamine levels in serum. 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 is proportional to the concentration of fructosamine in samples and the increase in absorbance (OD 530nm) can be monitored using a spectrophotometer. The kit includes a Thiol Blocking Reagent and a Sample Cleaning Mix that minimizes the interference of other endogenous reducing agents which ensures accurate measurements of Fructosamine in biological samples. The assay is simple, reproducible and can detect as low as 20 µmol/L of fructosamine in samples.

Applications

Measurement of Fructosamine levels in serum

Storage

Store the kit at -20°C. Briefly centrifuges small vials prior to opening. Read entire protocol before performing the assay.
Fructosamine Buffers A and B: Store at 4 °C. Bring to room temperature before use.
NBT: Aliquot and Store at -20°C. Protect from light. Bring to room temperature before use.
Thiol Blocking Reagent: Reconstitute with 400 µl Fructosamine Buffer A. Vortex and Mix Well. Store at -20°C. When dissolved, use within 2 month.
Sample Cleaning Mix: Reconstitute with 1 ml Fructosamine Buffer A. Allow contents to dissolve intensively. Aliquot and keep at -20°C. When dissolved, use within 2 month.
Fructosamine Calibrator: Upon received, store at 4°C. Stable for 2 months at 4 °C after opening.

Size

100 assays

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Kit Components

Fructosamine Buffer A; 25 ml
NBT (in DMF); 580 µl
Thiol Blocking Reagent; 1 vial
Sample Cleaning Mix (Lyophilized); 1 vial
Fructosamine Buffer B; 40 ml
Fructosamine Calibrator (3.2 mM); 180 µl

Materials Required but Not Supplied

96-well clear plate with flat bottom
Multi-well spectrophotometer

Assay Protocol

1. Sample and Fructosamine Calibrator Preparation: Add 10 µl of undiluted samples or 10 µl of dH2O into wells of a clear 96-well plate and label as "Sample" and "Background". For positive control, add 3 µl of Fructosamine Calibrator into well(s), adjust the volume to 10 µl with Fructosamine Buffer A and label as "Fructosamine Calibrator(s)".
Note: 3 µl of Fructosamine Calibrator is equivalent to 9.6 nmol Fructosamine (see step 6).
2. Thiol Blocking Reagent and Sample Cleaning Mix Preparation: Prepare Reagent Mix by adding 2 µl of Reconstituted Thiol Blocking Reagent, 5 µl of Reconstituted Sample Cleaning Mix with 30 µl Fructosamine Buffer A (total volume is 37 µl/per well). Prepare enough reagents for the number of samples to be assayed. Add Prepared Reagent Mix (37 µl) to all wells containing "Sample", "Background" and "Fructosamine Calibrator". Mix well.
Note: The test is very sensitive to temperature; pre-warm Fructosamine Buffer A to 37 °C before adding to well(s).
3. NBT Preparation: Add 3 µl NBT to each wells containing "Sample", "Background" and "Fructosamine Calibrator". Partial volume of each well should be 50 µl. Mix well. Pre-Incubate the plate at 37 °C for 10 min to remove interferences, avoid light.
4. Fructosamine Reaction: Add 200 µl of Fructosamine Buffer B to each well containing the "Sample", "Background", "Fructosamine Calibrator". Mix well. Total volume in every well should be 250 µl. Incubate the plate at 37°C for 5 min, avoid light.

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Notes: 1) The test is very sensitive to temperature; pre-warm Fructosamine Buffer B to 37 °C before adding to well(s).
2) We suggest use a multichannel pipette to assay multiple samples/wells.
5. Measurement: After 5 min. incubation, measure absorbance 530 nm at 37°C at two specific time points (t1=5 min, OD1 and t2=15 min, OD2).
Note: The first 5 minutes of incubation (after the addition of Fructosamine Assay Buffer B) minimizes the effect of non-specific reducing substances for calculating Fructosamine levels. Do not use the first 5 min OD 530 nm readings for calculating fructosamine concentrations.
6. Calculation: Calculate the change in absorbance during the 10 min interval (A=OD2-OD1) of each wells labeled as Sample, Calibrator and Background. Subtract "Background" readings from "Sample" and from "Calibrator", respectively.
Fructosamine amounts in sample = [A(sample) - A(background)]/[A(calibrator) - A(background)] *9.6 = B (nmol)
Fructosamine concentration in sample= B/V (nmol/ml, μM)
Where: B = Fructosamine amount (nmol)
V = the sample volume added into reaction well (ml)
9.6= Fructosamine amounts (nmol) of 3 μl of Fructosamine Calibrator

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