Fructosamine Content Assay Kit (ELISA)

Cat. No. KITG-087 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Glycosylated serum protein (GSP), also known as fructosamine, is a high molecular weight ketoamine structure formed during the non-enzymatic glycation process of plasma proteins with glucose. The concentration of GSP is positively correlated with blood glucose levels and remains relatively stable over time, reflecting the average blood glucose level over a period of 1-3 weeks. This makes GSP a valuable clinical indicator for distinguishing stress-induced hyperglycemia from sustained hyperglycemia and for evaluating the overall control status of diabetes mellitus. The assay principle is based on the non-enzymatic glycation reaction between serum glucose and the N-terminal amino groups of albumin and other serum protein molecules, forming high molecular weight ketoamine structures. Under alkaline conditions, these ketoamine structures react with nitroblue tetrazolium (NBT) to generate a purple-red formazan compound. The resulting product exhibits a characteristic absorption peak at 530 nm, and the GSP content can be quantitatively determined by measuring the change in absorbance at this wavelength.
Description	This assay kit utilizes microplate reader-based spectrophotometry for the quantitative determination of glycosylated serum protein (GSP) / fructosamine content in serum or plasma samples. The assay is based on the non-enzymatic glycation reaction between serum glucose and the N-terminal amino groups of albumin and other serum proteins, forming high molecular weight ketoamine structures. Under alkaline conditions, these ketoamine structures react with nitroblue tetrazolium (NBT) to produce a purple-red formazan compound. The resulting product exhibits a characteristic absorption peak at 530 nm, allowing quantitative detection of GSP content through absorbance measurement using a microplate reader or

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	spectrophotometer. Detection Method: Microplate Reader / Spectrophotometry. Compatible Sample Types: Serum, Plasma. Storage: 2-8°C. Size: 100T/96S. Usage: For Research Use Only. Not for Use in Diagnostic Procedures. Detection Wavelength: 530 nm. Standard Diluent Concentration: 4 mmol/L.
Form	Kit
Applications	Distinguishing stress-induced hyperglycemia. Evaluating diabetes control status. Reflecting average blood glucose levels over 1-3 weeks. Quantitative determination of glycosylated serum protein (GSP) / fructosamine content in serum or plasma samples.
Notes	Materials Required but Not Supplied Visible spectrophotometer or microplate reader. Micro glass cuvette with a 10 mm path length or a 96-well microplate. Adjustable pipette. Benchtop centrifuge. Constant-temperature water bath or incubator. Distilled water. Technical Notes 1. After completion of the 37°C color development, Reagent 4 should be added immediately. It is recommended not to process too many samples at one time to prevent delayed addition of Reagent 4, which may affect the assay results. 2. If A_sample is greater than 1.0 or ΔA_sample is greater than 0.8, appropriately dilute the sample before measurement. If the measured absorbance is low or close to the blank value, increase the sample volume during sample preparation and re-extract before measurement; adjust the calculation accordingly. 3. To ensure accurate results and avoid reagent loss, read the instructions carefully before measurement to confirm that reagent storage and preparation are adequate and that the operating steps are clear. Perform a pilot assay with 2-3 samples with expected significant differences. Sample Preparation For serum (plasma) and other liquid samples: Collect serum (plasma) into a centrifuge tube. Treat the sample at a ratio of serum (plasma) volume : Reagent 1 volume = 10:1. It is recommended to transfer 0.5 mL serum (plasma) into a centrifuge tube and add 0.05 mL Reagent 1. Mix thoroughly and incubate at 37°C for 30 min. This is the sample to be tested. Standard Application Solution Preparation (prep

	are fresh before use) Prepare at a volume ratio of standard diluent : Reagent 1 = 10: 1. Incubate at 37°C for 30 min before use. Mix thoroughly. This is the standard applic ation solution. Assay Protocol 1. Preheat the spectrophotometer or microplate reader for more than 30 min, set the wavelength to 530 nm, and zero with distilled water. 2. Prepare the standard application solution immediately before use by mixing the stand ard diluent and Reagent 1 at a volume ratio of 10:1. Incubate at 37°C for 30 min befor e use and mix thoroughly. 3. Add the following reagents sequentially into a 96-well mi croplate or centrifuge tubes: Sample Group: Add 10 µL sample to be tested and 200 µL Reagent 3. Mix thoroughly. Control Group: Add 10 µL distilled water and 200 µL R eagent 3. Mix thoroughly. Standard Group: Add 10 µL standard application solution a nd 200 µL Reagent 3. Mix thoroughly. Blank Group: Add 10 µL Reagent 2 and 200 µL Reagent 3. Mix thoroughly. Incubate all groups at 37°C for 15 min for color developm ent. Then add 10 µL Reagent 4 to each group and mix thoroughly. Absorbance Meas urement Measure the absorbance at 530 nm. Record the values as A_sample, A_con trol, A_standard, and A_blank. Calculate ΔA_sample = A_sample - A_control and ΔA_ standard = A_standard - A_blank. The control group and blank group only need to be measured 1-2 times. Assay Time Spectrophotometer or microplate reader preheating: >30 min. Sample incubation: 30 min at 37°C. Standard application solution preparatio n: 30 min at 37°C. Color development: 15 min at 37°C. Analysis Glycosylated Serum Protein (GSP) Content Calculation: GSP Content (mmol/L) = (C_standard × ΔA_samp le × D) / ΔA_standard = (4 × ΔA_sample × D) / ΔA_standard. C_standard: Concentrati on of standard diluent, 4 mmol/L. D: Dilution factor of the sample to be tested. ΔA_sa mple = A_sample - A_control. ΔA_standard = A_standard - A_blank. If the sample ha s been diluted during preparation, multiply the calculated result by the corresponding dilution factor.
Storage	Kit Components and Storage Reagent 1: Liquid, 5 mL × 1 vial; store at 4°C. Reagent 2: Liquid, 2 mL × 1 vial; store at -20°C. Reagent 3: Liquid, 30 mL × 1 vial; store at 4°C. Reagent 4: Liquid, 2 mL × 1 vial; store at 4°C. Standard: Powder, 1 vial; store at

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	-20°C. Prior to use, add 800 µL Reagent 2 and dissolve thoroughly to obtain a 10 mmol/L DMF standard solution. Overall Kit Storage: 2-8°C.
Reconstitution	Standard and Working Solution Preparation 1. Standard Solution: Prior to use, add 800 µL Reagent 2 to the Standard and dissolve thoroughly. This yields a 10 mmol/L DMF standard solution. 2. Standard Diluent Preparation (prepare fresh before use): Dilute the 10 mmol/L DMF standard solution with Reagent 2 to 4 mmol/L and mix thoroughly. This is the standard diluent. 3. Standard Application Solution Preparation (prepare fresh before use): Mix the standard diluent and Reagent 1 at a volume ratio of 10:1. Incubate at 37°C for 30 min before use and mix thoroughly.
Reaction conditions	Preheat the spectrophotometer or microplate reader for more than 30 min, set the wavelength to 530 nm, and zero with distilled water. Prepare the sample by mixing serum or plasma with Reagent 1 at a 10:1 volume ratio and incubating at 37°C for 30 min. Prepare the standard application solution by mixing standard diluent and Reagent 1 at a 10:1 volume ratio and incubating at 37°C for 30 min. For the assay, add 10 µL sample, distilled water, standard application solution, or Reagent 2 to the corresponding group, followed by 200 µL Reagent 3. Incubate at 37°C for 15 min, add 10 µL Reagent 4 immediately, mix thoroughly, and measure absorbance at 530 nm.
Unit definition	Glycosylated Serum Protein (GSP) Content Calculation: GSP Content (mmol/L) = (C_standard × ΔA_sample × D) / ΔA_standard = (4 × ΔA_sample × D) / ΔA_standard. C_standard is the concentration of the standard diluent, 4 mmol/L. D is the dilution factor of the sample to be tested. ΔA_sample = A_sample - A_control. ΔA_standard = A_standard - A_blank. If the sample has been diluted during preparation, multiply the calculated result by the corresponding dilution factor.
Usage	For Research Use Only. Not for Use in Diagnostic Procedures.

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