Catalog #80109

V.2

Crystal Chem

Glycated Serum Protein (GSP) Kit Instructions

For the quantitative determination of glycated serum proteins in human serum or plasma

Catalog #80109
96 Assays

For research use only. Not for use in diagnostic procedures.

Crystal Chem, Inc.
1536 Brook Drive, Suite A
Downers Grove, IL 60515, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
URL: http://www.crystalchem.com

Catalog #80109

TABLE OF CONTENTS

A. Intended Use

The Glycated Serum Protein (GSP) kit is for the quantitative determination of glycated serum proteins in human serum or plasma. The glycated serum protein concentration using the assay is expressed as μmol/L. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Studies show that the measurement of glycated serum proteins is useful for monitoring diabetic patients. In diabetic patients, elevated blood glucose levels correlate with increased glycated serum proteins. Glycated serum protein levels in serum is a medium term indicator of diabetic control (2-3 weeks). Glycated serum proteins are used to measure the glycation gap for diagnosis of complications and nephropathy in diabetic patients.

Recent studies showing improved patient outcomes and better short term indications of glycemic control have stimulated intensive research on glycated serum protein levels. As a result, the accurate measurement of glycated serum proteins in experimental animals is becoming increasingly important.

C. Principle of the Assay

The GSP kit uses proteinases to digest serum proteins into low molecular weight glycated protein fragments, and uses specific fructosaminase to yield glucosone and H_2O_2. The H_2O_2 released is measured by a colorimetric reaction. The absorbance at 570 nm is proportional to the concentration of glycated serum protein (μmol/L).

D. Kit Storage

1. Upon receipt of the GSP kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
CC1	Reagent 1 (liquid)	1 X 23 mL
CC2	Reagent 2 (liquid)	1 X 7.5 mL
CAL1	Calibrator 1 (lyophilized)	1 X 1 mL

Note: Optional controls are available separately (Cat# 80103)

E.2. Materials required but not provided

Microplates
Micropipettes and disposable tips
Clean glass tubes and test tube racks
Volumetric flasks
Incubator (37ºC)
Distilled water
Microplate reader or spectrophotometer or chemistry analyzer (should read A_{570} values)
0.9% saline

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (20 µL), pipetting should be done as carefully as possible. A high quality 20 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Serum: Collect blood, allow to clot, and centrifuge for 20 min at 2,000 x *g*.

Plasma: Collect blood into a tube containing EDTA (final concentration: 0.1%), and centrifuge for 20 min at 2000 x *g*.

It is recommended that samples be used within 2 weeks of collection when stored refrigerated. If assay is to be performed more than 2 weeks after collection, samples should be frozen.

I. Assay Procedure

I.1. Preparation of reagents

All reagents are provided ready-to-use and should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

I.2. Preparation of samples, calibrators, and controls

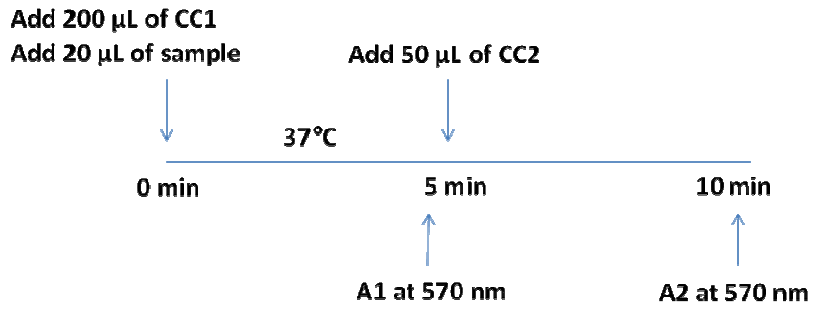
1. Glycated Serum Protein Calibrator 1 (marked as “CAL1”) is provided in lyophilized form and must be reconstituted with 1.0 mL of distilled water. To ensure complete reconstitution, equilibrate vials at room temperature for 30 minutes before first use.
   Note: Reconstituted calibrators are stable for 14 days when capped tightly and stored at 2-8°C. In addition to running the calibrators provided, the assay requires running a blank calibrator. 0.9% saline should be used for running the blank calibrator.
2. Bring all samples, calibrators, and controls to room temperature. Frozen samples should be allowed to fully thaw before proceeding.
3. Prior to testing, serum samples, calibrators, and controls should be thoroughly mixed by gentle inversion.

I.3. Glycated Serum Protein (µmol/L) assay procedure

The procedure below reflects a manual procedure performed using a microplate and a microplate reader (ideal when running multiple samples). The procedure can be easily adopted as needed to be run in a glass tube with a spectrophotometer. The assay can also be adopted to work on various automated analyzers. Please contact Crystal Chem for more information.

1. Add 200 µL of Reagent CC1 into each well (as needed) of a microplate and mix well by repeated pipetting.
2. In each well, add 20 µL of sample, calibrator, or control and mix well by repeated pipetting.
3. Place microplate in incubator (37ºC) and allow microplate to equilibrate to 37ºC over 5 minutes.
4. Measure absorbance using a plate reader (measure A_{570} values).
   Note: The GSP assay is an end-point assay and the first reading point A1 is right before the addition of reagent CC2.
5. Add 50 µL of Reagent CC2 and mix well by repeated pipetting.
6. Measure the increase in absorbance after 5 minutes at 37ºC using a plate reader (measure A_{570} values).

Figure 1. Summary of assay procedure



I.4. Determining the Glycated Serum Protein (µmol/L) concentration

1. Calculate the change in absorbance ΔA (300sec ~ 600sec)

   $\Delta A = (OD_{570nm,\ 600sec}) - (OD_{570nm,\ 300sec})$

2. Using linear graph paper, construct the glycated serum protein calibration curve by plotting the mean change in absorbance value for each calibrator (including the zero calibrator) on the Y axis versus the corresponding calibrator glycated serum protein concentration on the X axis.
   Note: *Calibrator values vary per lot and should be obtained from the calibrator label. A calibration curve should be plotted every time the assay is performed.*
3. Glycated serum protein concentrations in the samples are interpolated using the calibration curve and mean change in absorbance values for each sample. The glycated serum protein concentration is expressed in µmol/L.
   Note: *Samples with high glycated serum protein concentrations (1,300.0 µmol/L or higher) should be not be diluted and rerun. Instead the values should be reported as higher than 1,300.0 µmol/L (>1,300.0 µmol/L).*

J. Performance characteristics

J.1. Assay range

The Glycated Serum Protein (GSP) kit has a linear range from 20 - 1,300 µmol/L.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

Crystal Chem Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product.

THERE IS NO WARRANTY OF MERCHANTABILITY OF THE PRODUCTS, OR THAT SUCH PRODUCTS ARE FIT FOR ANY PARTICULAR PURPOSE. CRYSTAL CHEM INC'S LIABILITY SHALL NOT EXCEED THE RETURN OF THE PURCHASE PRICE, AND UNDER NO CIRCUMSTANCES SHALL CRYSTAL CHEM INC. BE LIABLE FOR SPECIAL OR CONSEQUENTIAL DAMAGES, OR EXPENSES ARISING DIRECTLY OR INDIRECTLY FROM THE USE OF THIS PRODUCT.