

Glycoprotein Carbohydrate Estimation Kit

23260

0754w

Product Description

Number

23260

Description

Glycoprotein Carbohydrate Estimation Kit

Kit Components:

Sufficient materials are provided in the kit to perform 250 microwell plate assays or 60 test tube assays.

Sodium meta-periodate, 500 mg**Glycoprotein Detection Reagent, 500 mg****Glycoprotein Assay Buffer, 250 ml****Caution:** Contains 0.1% sodium azide

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Lyophilized Protein Standards

- A. **Negative Controls, Lysozyme, 2.5 mg; Bovine serum albumin, 2.5 mg**
- B. **Positive Controls, Ovalbumin, 2.5 mg; Human apotransferrin, 2.5 mg; Fetuin, 0.25 mg; α_1 -Acid glycoprotein, 0.25 mg**

Store all components at 4°C

Introduction

Glycoproteins are widely distributed in nature and serve a wide variety of functions. They are found in blood and secretions, in cell membranes, and in connective tissues. Glycoproteins consist of carbohydrate moieties covalently linked to a polypeptide backbone. They represent a diverse group, ranging from substances in which the carbohydrate component represents less than 1% of the total weight to those in which it represents over 80% of the total weight. The sugars that commonly occur in glycoproteins include galactose, mannose, glucose, *N*-acetylglucosamine, *N*-acetylgalactosamine, sialic acid, fucose, and xylose.

Pierce's Glycoprotein Carbohydrate Estimation Kit is designed for the detection of glycoproteins and for the estimation of carbohydrate content in glycoproteins.

Principle of the Pierce Glycoprotein Carbohydrate Estimation Kit

Glycoprotein is oxidized with sodium meta-periodate and the aldehyde formed is detected using the proprietary Glycoprotein Detection Reagent. The Glycoprotein Detection Reagent gives off a purple color with aldehyde with an absorption maximum at 550 nm. The absorbance at 550 nm is proportional to the percentage of carbohydrate component in glycoproteins. Proteins that are not glycosylated, like lysozyme and bovine serum albumin, give very low absorbance at 550 nm. The assay is rapid and simple.

Note: Please read this instruction booklet completely before beginning the assay.

Materials Required, but not Supplied

1 N NaOH (required to dissolve the Glycoprotein Detection Reagent)

96-Well EIA Plates (Prod. No. 15041) or 8-Well EIA Strip Plates (Prod. No. 15031), required to perform the assays in microwell plate format

15 x 100 mm test tubes, required to perform the assays in test tube format

Microwell plate reader that can read absorbance at 550 nm or UV/Visible Spectrophotometer that can measure absorbance at 550 nm

Make Ready

1. Bring Glycoprotein Carbohydrate Estimation Kit to room temperature.
2. Prepare a 10 mM solution of sodium meta-periodate by dissolving 21.4 mg of sodium meta-periodate in 10 ml of Glycoprotein Assay Buffer.
Note: This solution must be freshly prepared just prior to performing the assay.
3. Prepare a 0.5% solution of Glycoprotein Detection Reagent in 1 N NaOH by dissolving 50 mg of Glycoprotein Detection Reagent in 10 ml of 1 N NaOH. Depending upon the number of assays to be performed, the volume of the preparation of Glycoprotein Detection Reagent can be increased or decreased.

Note: This solution must be freshly prepared just prior to performing the assay.

4. Reconstitute the lyophilized protein standards.

Note: During shipment, the lyophilized proteins may come in contact with the grey vial septa. Before opening, check to see that the lyophilized protein is settled to the bottom of each vial. If necessary, tap the side of the vial gently to settle the lyophilized protein. Carefully remove the septa to avoid disturbing any protein that may have settled on the underside of the septa. Add 1 ml of Glycoprotein Assay Buffer to the vial. Carefully return the septa into the vial opening. Holding the septa firmly in place with your thumb, dissolve the protein by gently rocking the vial so that the buffer contacts all inside surfaces.

These protein standard solutions can be stored at 4°C for at least one month without affecting the performance of the assay.

5. Dissolve the sample to be assayed in Glycoprotein Assay Buffer at concentrations of 0.25 and 2.5 mg/ml. If the sample is already in solution, dilute the sample in Glycoprotein Assay Buffer at concentrations of 0.25 and 2.5 mg/ml.

Assay Protocols

Protocol for Microwell Plate Format

Note: Perform the assay in triplicate.

1. Place 50 µl of each of the standards and the sample in the wells of a microwell plate. For the blank, use 50 µl of Glycoprotein Assay Buffer.
2. Add 25 µl of 10 mM sodium meta-periodate to each well.
3. Mix the the plate for 30 seconds in a microwell plate shaker and incubate at room temperature for 10 minutes.
4. Add 150 µl of the 0.5% Glycoprotein Detection Reagent to each well.
5. Mix the plate for 30 seconds in a microwell plate shaker.
6. Incubate the plate at room temperature for 1 hour.
7. Read the optical density at 550 nm in a microwell plate reader.
8. Plot a standard curve.

Protocol for Test Tube Format

Note: Perform the assay in duplicate.

1. Place 0.2 ml of each of the standards and the sample in test tubes. For the blank, use 0.2 ml of Glycoprotein Assay Buffer.

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2. Add 0.1 ml of 10 mM sodium meta-periodate to each tube.
3. Vortex to mix; then incubate at room temperature for 10 minutes.
4. Add 0.3 ml of 0.5% Glycoprotein Detection Reagent to each tube.
5. Vortex to mix; then incubate the tubes at room temperature for 1 hour.
6. Read the optical density at 550 nm in a spectrophotometer.
7. Plot a standard curve.

Results

A typical assay in microwell plate format using Glycoprotein Carbohydrate Estimation kit is shown in Table 1

Table 1

Glycoprotein Detection and Estimation in Microwell Plate Format Using Glycoprotein Carbohydrate Estimation Kit

Protein	Protein Concentration (mg/ml)	Total Carbohydrate Content (%)	Absorbance at 550 nm
Blank	0	0	0.109
Lysozyme	2.5	0	0.171
Bovine serum albumin	2.5	Trace	0.188
Ovalbumin	2.5	3.2	0.294
Apo-Transferrin	2.5	5.8	0.517
Fetuin	0.25	22.9	0.363
	2.5	22.9	3.63 (Calculated)
α_1 -Acid Glycoprotein	0.25	41.4	0.447
	2.5	41.4	4.47(Calculated)

Table 2

Glycoprotein Detection and Estimation in Test Tube Format Using Glycoprotein Carbohydrate Estimation Kit

Protein	Protein Concentration (mg/ml)	Total Carbohydrate Content (%)	Absorbance at 550 nm
Blank	0	0	0.107
Lysozyme	2.5	0	0.214
Bovine serum albumin	2.5	Trace	0.256
Ovalbumin	2.5	3.2	0.434
Apo-Transferrin	2.5	5.8	0.753
Fetuin	0.25	22.9	0.526
	2.5	22.9	5.26 (Calculated)
α_1 -Acid Glycoprotein	0.25	41.4	0.610
	2.5	41.4	6.1 (Calculated)

It is evident from Tables 1 and 2 that the Pierce Glycoprotein Carbohydrate Estimation Kit can be used for the rapid detection of glycoproteins. From the absorbance values at 550 nm of the sample and the standards of known carbohydrate content, it is possible to estimate the carbohydrate content of the sample.

Table 3 can be used for data analysis and for the estimation of carbohydrate content in the sample.

Table 3

Estimation of Carbohydrate Content in the Sample Using Glycoprotein Carbohydrate Estimation Kit

Protein	Protein Concentration (mg/ml)	Total Carbohydrate Content (%)	Absorbance at 550 nm
Blank	0	0	
Lysozyme	2.5	0	
Bovine serum albumin	2.5	Trace	
Ovalbumin	2.5	3.2	
Apo-Transferrin	2.5	5.8	
Fetuin	0.25	22.9	
	2.5	22.9	
α_1 -Acid Glycoprotein	0.25	41.4	
	2.5	41.4	
Sample	0.25		
	2.5		

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