INSTRUCTIONS

Glycoprotein Carbohydrate Estimation Kit

23260

0754.3

Number	Description
23260	**Glycoprotein Carbohydrate Estimation Kit**, sufficient reagents for 250 microplate assays or 60 test tube assays
	Kit Contents:
	Sodium *meta*-periodate, 500mg
	Glycoprotein Detection Reagent, 500mg
	Glycoprotein Assay Buffer, 250mL, contains 0.1% sodium azide
	Negative Controls: lysozyme, 2.5mg; bovine serum albumin, 2.5mg
	Positive Controls: ovalbumin, 2.5mg; human apotransferrin, 2.5mg; fetuin, 0.25mg; α_1-acid glycoprotein, 0.25mg

Introduction

The Thermo Scientific Glycoprotein Carbohydrate Estimation Kit is a simple, rapid method for glycoprotein detection and carbohydrate content estimation. Glycoprotein is first oxidized with sodium *meta*-periodate to form aldehydes that react with the proprietary Glycoprotein Detection Reagent. The resulting purple reaction has an absorption maximum at 550nm that is proportional to the carbohydrate content in the glycoprotein. Unknowns are compared with protein standards of known glycoprotein content (see the Additional Information Section). Non-glycosylated proteins, such as lysozyme and bovine serum albumin, produce a low absorbance at 550nm.

Additional Materials Required

- 1N NaOH (used to dissolve the Glycoprotein Detection Reagent)
- 96-well microplate (Product No. 15041 or 15031), if using the microplate format
- 15 × 100mm test tubes, if using the test tube format
- Microplate reader or UV/visible spectrophotometer that can measure absorbance at 550nm

Material Preparation

Note: Equilibrate the Glycoprotein Carbohydrate Estimation Kit components to room temperature before use.

Sodium *meta*-periodate Solution	Immediately before use, prepare 10mM sodium *meta*-periodate by dissolving 21.4mg of sodium meta-periodate in 10mL of Glycoprotein Assay Buffer.
Glycoprotein Detection Reagent	Immediately before use, prepare 0.5% Glycoprotein Detection Reagent by dissolving 50mg of the reagent in 10mL of 1N NaOH.
Glycoprotein sample	Dissolve sample in Glycoprotein Assay Buffer at 0.25 and 2.5mg/mL. If the sample is already in solution, dilute sample in Glycoprotein Assay Buffer at 0.25 and 2.5mg/mL. **Note:** Tris interferes with carbohydrate detection. For Tris-containing samples, perform a buffer exchange using the Glycoprotein Assay Buffer and a desalting column (e.g., Product No. 89882 for the microplate procedure or 89889 for the test tube procedure).
Protein standards	During shipment, lyophilized proteins have may come in contact with the septa. Before opening, verify that protein is settled to the bottom of each vial. If necessary, gently tap the vial sides to settle protein. Carefully remove septa to avoid disturbing any protein that may have settled on its underside. Add 1mL of Glycoprotein Assay Buffer to each protein standard vial. Replace septa and gently rock vial so that the buffer contacts all inside surfaces. Store reconstituted standard solutions for up to one month at 4°C.

Microplate Procedure

1. Place 50µL of each standard and the sample in the plate wells. For the blank, use 50µL of Glycoprotein Assay Buffer. Test each sample and standard in triplicate.
2. Add 25µL of the sodium *meta*-periodate solution to each well.
3. Mix plate for 30 seconds in a microplate shaker.
4. Cover and incubate plate at room temperature for 10 minutes.
5. Add 150µL of the Glycoprotein Detection Reagent to each well.
6. Mix plate for 30 seconds in a microplate shaker.
7. Cover and incubate plate at room temperature for 1 hour.
8. Measure absorbance at 550nm in a microplate reader and plot a standard curve.

Test Tube Procedure

1. Place 0.2mL of each standard and the sample in test tubes. For the blank, use 0.2mL of Glycoprotein Assay Buffer. Test each sample and standard in duplicate.
2. Add 0.1mL of the sodium *meta*-periodate solution to each tube and vortex to mix.
3. Cover and incubate tubes at room temperature for 10 minutes.
4. Add 0.3mL of Glycoprotein Detection Reagent to each tube and vortex to mix.
5. Cover and incubate tubes at room temperature for 1 hour.
6. Measure the absorbance at 550nm in a spectrophotometer and plot a standard curve.

Additional Information

A. Protein Standard Data

The absorbance values at 550nm can be used to estimate carbohydrate content of the sample by comparing to standards of known carbohydrate content. Typical standard values for the microplate and test tube formats are reported in Tables 1 and 2, respectively.

Table 1. Values for protein standards of known carbohydrate content using the Glycoprotein Carbohydrate Estimation Kit in the microplate format.

Protein	Protein (mg/mL)	Carbohydrate Content (%)	Absorbance (550nm)
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
Fetuin	2.5	22.9	3.63 (Calculated)*
α_1-Acid Glycoprotein	0.25	41.4	0.447
α_1-Acid Glycoprotein	2.5	41.4	4.47 (Calculated)*

*The 2.5mg/mL standard values for proteins with greater than 10% carbohydrate content is calculated by multiplying the absorbance value obtained for the 0.25mg/mL standard or sample by 10.

Table 2. Values for protein standards of known carbohydrate content using the Glycoprotein Carbohydrate Estimation Kit in the test tube format.

Protein	Protein (mg/mL)	Carbohydrate Content (%)	Absorbance (550nm)
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
Fetuin	2.5	22.9	5.26 (Calculated)*
α_1-Acid Glycoprotein	0.25	41.4	0.610
α_1-Acid Glycoprotein	2.5	41.4	6.1 (Calculated)*

*The 2.5mg/mL standard values for proteins with greater than 10% carbohydrate content is calculated by multiplying the absorbance value obtained for the 0.25mg/mL standard or sample by 10.

B. Template for data analysis and estimation of carbohydrate content in a sample

Protein	Protein (mg/mL)	Carbohydrate Content (%)	Absorbance (550nm)
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	
Fetuin	2.5	22.9	
α_1-Acid Glycoprotein	0.25	41.4	
α_1-Acid Glycoprotein	2.5	41.4	
Sample	0.25		
Sample	2.5		

Related Thermo Scientific Products

23259 **Lyophilized Glycoprotein Standards Set,** includes negative and positive controls

24562 **Glycoprotein Staining Kit,** contains sufficient materials to stain 10 mini gels or 20 nitrocellulose membranes (8 × 8cm)

24590 **GelCode™ Blue Stain Reagent,** for the total protein stain of 25 SDS-PAGE mini gels

24565 ***O*-GlcNAc Western Blot Detection Kit,** contains sufficient reagents for approximately 10 mini-blots

Product Reference

1. Huang, F. *et al*. (1999). Isolation, purification and characterization of pregnancy-specific protein B from elk and moose placenta. *Biology of Reproduction*. **61:**1056-61.

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