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A Geno Technology, Inc. (USA) brand name

PhosphoQuant™

For Phosphoprotein Phosphate Estimation

(Cat. #786-256)

INTRODUCTION

Phosphorylation and dephosphorylation of proteins perform a significant regulatory role in a variety of cellular processes. Therefore, to detect changes in the phosphorylation status of proteins has become a very important parameter in cell biology / proteomics research. *Phospho*Quant™ provides a simple procedure for estimation of phosphoproteins. It can also be used for the determination of the amount of a purified preparation of a known phosphoprotein in a sample. The *Phospho*Quant assay is based on the alkaline hydrolysis of phosphate from seryl and threonyl residues in phosphoprotein. The released phosphate is quantitated by using the *Phospho*-Green dye provided with the kit.

ITEM(S) SUPPLIED (Cat. # 786-256)

Description	Size
Phospho-Green Dye	50 ml
Phospho-Green Dilution Buffer	50 ml
Phospho-Green Stabilizer	5 ml
PhosphoQuant Assay Buffer [10X]	25 ml
Phosphate Standard (300µM)	10 ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store the kit components at 4°C, upon arrival. When stored and used properly, this kit is good for 1 year.

ADDITIONAL ITEMS REQUIRED:

- 2.0N NaOH
- 4.5N HCl
- Assay tubes or 96-well plate
- Spectrophotometer and microplate reader.

  NOTE: *All glassware, tubes etc used in this assay must be clean and phosphate free.*

PREPARATION BEFORE USE

- Warm up all reagents to room temperature.
- Dilute 10X *Phospho*Quant Assay Buffer to 1X with deionized water.
- Prepare phosphate standard set of concentrations (0-30µM) as indicated in the following table:

Final Concentration [µM]	Phosphate Standard (µl)	1X *Phospho*Quant Assay Buffer (µl)
0	0	1000
5	16.7	983.3
10	33.3	966.7
15	50	950
20	66.7	933.3
25	83.3	916.7
30	100	900

- Prepare or dilute your protein sample ~ 4-40µg/ml with 1X Assay Buffer.
- Preparation of *Phospho*-Green reagent: Mix equal volumes of the *Phospho*-Green Dye and *Phospho*-Green Dilution Buffer. Add 50µl *Phospho*-Green Stabilizer per ml *Phospho*-Green reagent and mix well. The *Phospho*-Green reagent must be prepared fresh before performing the assay. Ideally use the *Phospho*-Green reagent in 30 minutes after preparation.

PROTOCOL

1ml Assays

Perform the assay in triplicate.

1. Add 250µl of each dilution of the Phosphate Standard and the protein sample to a 1.5ml test tube. Use 250µl 1X Assay Buffer as blank.
2. Add 250µl 2.0N NaOH to each tube and mix. Incubate the tubes with protein sample for 30 minutes at 65°C (in an oven or a water bath).
3. Add 250µl 4.5N HCl to each tube and mix.
4. Add 250µl *Phospho*-Green reagent to each tube. Vortex briefly and incubate for 5 minutes at room temperature.
5. Read the absorbance at 620-640nm in a spectrophotometer.
6. Plot absorbance against Phosphate Standard concentration and calculate the moles of phosphate per mole of protein as described below.

Micro-Assays

Perform the assay in triplicate.

NOTE: *Because of the nature of rapid reaction and mixing limitation when working with microplate, the best results can be obtained by performing the reaction in test tubes.*

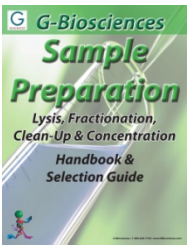
1. Add 50µl of each dilution of the Phosphate Standard and the protein sample to a 0.5ml or 1.5ml test tube. Use 50µl 1X Assay Buffer as blank.
2. Add 50µl 2.0N NaOH to each tube. Vortex it briefly to mix. Incubate the tubes with protein sample for 30 minutes at 65°C (in an oven or a water bath).
3. Add 50µl 4.5N HCl to each tube and vortex briefly to mix.
4. Add 50µl *phospho*-green reagent to each tube. Vortex briefly and incubate for 5 minutes at room temperature. Transfer 150µl from each tube to each well of the microplate.
5. Read absorbance at 620-640nm in a microplate reader.
6. Plot absorbance against Phosphate Standard concentration and calculate the moles of phosphate per mole of protein as described below.

Calculation of the moles of phosphate per mole of protein:

1. Plot a standard curve and determine the phosphate concentration of your protein sample.
2. Convert the diluted protein sample concentration (µg/ml) to µM by dividing by the molecular weight of the sample protein (MW) and multiplying 1000.
3. Divide the phosphate concentration (µM) by the protein concentration (µM). This will give you the number of moles of phosphate per moles of protein sample

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/

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