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A Geno Technology, Inc. (USA) brand name

FOCUS™ PhosphoRich™

For Phosphoprotein & Phosphopeptide Enrichment

(Cat. # 786-255)

INTRODUCTION

Phosphorylation of protein is a cornerstone of proteomic research, as it plays a significant role in signaling mechanism. Propagation of extracellular signal received at the plasma membrane is controlled by phosphorylation events. The FOCUS™ PhosphoRich™ ready to use kit allows enrichment of phosphorylated proteins. It also provides a simple way to isolate phosphopeptides from complex samples. The kit contains resin filled spin columns with resin binding capacity ~20mg of phosphorylated ovalbumin each column. The resin columns supplied with the kit can be re-used, if regenerated and stored properly.

ITEM(S) SUPPLIED (Cat. # 786-255)

Description	Size
Phospho-Lysis Buffer [1X]	25 ml
Phospho-Wash Buffer [10X]	25 ml
Phospho-Elution Buffer [5X]	25ml
Phospho-Column	5 Columns

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store at 4°C. When stored and used properly, the shelf life is 1 year.

BINDING CAPACITY

- 20mg phosphorylated ovalbumin/ column

ADDITIONAL ITEM(S) REQUIRED

Centrifuge, acetic acid, deionized water

OPTIONAL: *The Phospho-Lysis Buffer contains phosphatase inhibitors. If the inhibition of protease activity is required, add a cocktail of protease inhibitors (ProteaseArrest™, Cat. # 786-108 is recommended) to the Phospho-Lysis Buffer to prevent protease activities.*

PREPARATION BEFORE USE

Preparation of eukaryotic cell line proteins

1. Resuspend 50-100x10^6 cells thoroughly in Phospho-Lysis Buffer, using 0.1ml Phospho-Lysis Buffer per 1x10^6 cells.
2. Incubate for 10 minutes at room temperature.
3. Centrifuge 10,000x g for 10 minutes at 4°C. Discard the pellet.
4. Mix the protein solution with 0.2 volumes of 10X Phospho-Wash Buffer.
5. Incubate for 10 minutes at room temperature.
6. Centrifuge 10,000x g for 10 minutes at 4°C. Discard the pellet.

Preparation of other source proteins

1. Prepare the protein solution with the buffer of your choice or Phospho-Lysis Buffer.
2. Mix the protein solution with 0.2 volumes of 10X Phospho-Wash Buffer.
3. Incubate for 10 minutes at room temperature.
4. Check the pH and if >pH5, titrate the protein solution with 1% acetic acid to pH < 5.
5. Centrifuge at 10,000x g for 10 minutes at 4°C. Discard the pellet.

Preparation of phosphopeptides

1. Prepare phosphopeptide using a method of your choice.
2. Titrate the phosphopeptide solution with 1% acetic acid to pH < 5.

PROTOCOL

Phosphoprotein/ Phosphopeptide enrichment

1. Dilute an appropriate amount of 10X Phospho-Wash Buffer to 1X with deionized water.
2. Dilute an appropriate amount of 5 X Phospho-Elution Buffer to 1X with deionized water.
3. Equilibrate the Phospho-Column with 10ml 1X Phospho-Wash Buffer, allowing the buffer to pass through by gravity flow.
4. Load the prepared Phosphoprotein / Phosphopeptide solution to the column and allow to pass through by gravity flow.
5. Collect the flow through for downstream analysis.
6. Wash the column with 10ml 1X Phospho-Wash Buffer followed by 5ml deionized water.
7. Elute the bound Phosphoprotein / Phosphopeptide with 10ml Phospho-Elution buffer. The eluent can be collected in 1ml or smaller size fractions.
8. The eluent containing Phosphoprotein / Phosphopeptide is ready for the next step analysis.

NOTE*: The eluent contains salt concentrations that may not be suitable for 2D gel analysis. Dialyze the eluent against an appropriate buffer. We recommend using our Tube-O-DIALYZER. Alternatively, prepare and clean the sample with Perfect-FOCUS™ (Cat# 786-124) before 2D gel analysis.*

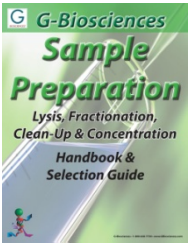
COLUMN REGENERATION

1. Column may be regenerated and used one more time.
2. For regeneration, wash the column with 10ml deionized water. Followed by 10ml 1% acetic acid.
3. Store the column in 1% acetic acid at 4°C. Equilibrate the column before use.

NOTE: *If the column is not stored and used properly, the binding capacity of the column will deteriorate.*

RELATED PRODUCTS

Download our Sample Preparation Handbook.



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

For other related products, visit our website at www.GBiosciences.com or contact us.

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