Phosphoprotein Enrichment Kit

Order No.: **0110/PPEK-KIT**

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CONTENTS:

Columns, prepacked (1 ml bed volume)	2 pieces
Washing Buffer (10 x, yellow, pH 5.5)	2 x 50 ml
Elution Buffer (10 x, red, pH 8.5)	1 x 50 ml
Lysis Buffer (1 x) for Cell/Tissue Extraction	2 x 50 ml
Sample Buffer (5 x, pink)	1 ml

The Lysis Buffer does not contain Phosphatase Inhibitors (may be added prior to use; do not interfere with purification). All buffer concentrates must be diluted with distilled water to prepare 1 x solutions. The KIT
does not contain 3%(v/v) acetic acid, which is required for reconstitution and storage of the column.

EXPERIMENTAL PROTOCOL:

It is important to use soluble protein for loading of the column at a pH < 5.5. A protein solution can be prepared from animal tissues or eucaryotic cell lines with the use of Lysis Buffer (1 x) according to the following protocols:

1) Preparation of Soluble Protein

a) from animal tissue:
Dilute animal tissue 1:4 with Lysis Buffer and homogenize.
Centrifuge (20 min, 10000g, 4 C), discard pellet.

b) from eucaryotic cell lines:
Resuspend cells in Lysis Buffer (1 ml Lysis Buffer/10E7 cells)
Incubate 5 min, RT
Centrifuge (10 min, 10000g, 4 C), discard pellet.

2) Column Preparation:
The column must be washed with 25 ml Washing Buffer (1 x) before loading.

Loading:
Mix the protein solution with 1/4 volume Washing Buffer (10 x, yellow), incubate for 30 min at RT.
Centrifuge the protein solution before loading (10 min, 10000 g) in order to remove proteins that have precipitated due to isoelectric precipitation. Discard pellet.
For loading, the pH of the sample must be <= 5.5. Please check pH before loading and titrate with acetic acid if necessary. If prominent precipitation occurs, lower pH to 4.5 - 5.5 using acetic acid and/or dilute protein solution 10fold. Centrifuge (10 min, 10000 g) again after titration and apply the
supernatant to the column in free flow.
The column will hold 20 mg phosphorylated ovalbumin maximum.

3) Washing:

Wash column in free flow with 50 ml Washing Buffer (1 x)

4) Elution:

Bound phosphoprotein is eluted with 10 x 1 ml Elution Buffer (1 x, red). Elution starts when the
colour of the eluate changes from yellow to red.
The resulting fractions are mixed 5:1 with Sample Buffer (5 x) and analysed by SDS-PAGE or Blot.

5) Reconstitution and Storage:

After use wash column with a) 50 ml H2O
b) 50 ml 3%(v/v) Acetic Acid

Do not treat the column with NaOH!
The column can be stored in 3%(v/v) acetic acid and reused twice.