

---

## Product Data Sheet

### Prod. No.: ALX-850-289-KI01 Phosphoprotein Enrichment Kit

---

This kit provides a quick and easy method for the enrichment of phosphoproteins from tissues or cell culture for immunoblotting. It contains 2 columns, wash buffer, elution buffer, lysis buffer, and SDS-PAGE sample buffer sufficient to process up to 25 mg of tissue or  $100 \times 10^7$  cells.

The binding capacity of each column is 20 mg of phosphorylated ovalbumin per use.

#### Kit contents:

Columns, prepacked, 1 ml bed volume.....	2 x 1 each
Wash buffer, 10X, yellow, pH 4.8 .....	2 x 50 ml
Elution buffer, 10X, red, pH 9.0 .....	1 x 50 ml
Lysis buffer, 1X, for cell/tissue extraction (100 mM NaCl, 20 mM NaF, 10 mM imidazole, 5 mM molybdate, 2 mM MgCl <sub>2</sub> , 2 mM vanadate, 0.2% TRITON® X-100, 0.1% NaN <sub>3</sub> , pH 6.8).....	2 x 50 ml
Sample buffer, 5X.....	1 x 1 ml

#### Notes:

All buffer concentrates must be diluted with distilled water to prepare 1X solutions.

Reconstitute and store columns in 3% acetic acid solution (not provided).

Lysis buffer contains phosphatase inhibitors, but no protease inhibitors (addition optional).

All protein samples used must have a pH of 5.5 or lower. Protein solutions can be prepared from animal tissues and/or eukaryotic cell lines with 1X lysis buffer as shown below.

#### Preparation of protein solution from animal tissue

1. Using a 1:4 tissue (mg):buffer (ml) ratio, homogenize the tissue with 1X lysis buffer.
  2. **Important:** Centrifuge the resulting sample at 10,000 x g for 20 minutes at +4°C. Discard pellet.
- 
-

### **Preparation of protein solution from eukaryotic cell lines**

1. Resuspend cells in 1 ml of 1X lysis buffer per  $10^7$  cells.
2. Incubate for 5 minutes at room temperature.
3. **Important:** Centrifuge resulting sample at 10,000 x g for 10 minutes at +4°C. Discard pellet.

### **Column Use**

1. Wash column with 25 ml of 1X wash buffer.
2. Mix the protein solution with 1/4 volume of 10X wash buffer and incubate for 30 minutes at room temperature.
3. Centrifuge (!) the protein solution at 10,000 x g for 10 minutes at +4°C. Discard pellet.
4. Confirm the sample is pH 5.5 or lower. Titrate with acetic acid if necessary.
5. Apply supernatant to the column in free flow. Note: The column can hold a maximum of 20 mg of phosphorylated ovalbumin.
6. Wash the column in free flow with 50 ml of 1X wash buffer and then 5 ml of H<sub>2</sub>O.
7. Elute bound phosphoprotein with 10 x 1ml of 1X elution buffer. Note: Elution begins when the color of the eluate changes from yellow to red.
8. Mix resulting fractions at a 5:1 ratio with 5X sample buffer and analyze by SDS-PAGE.

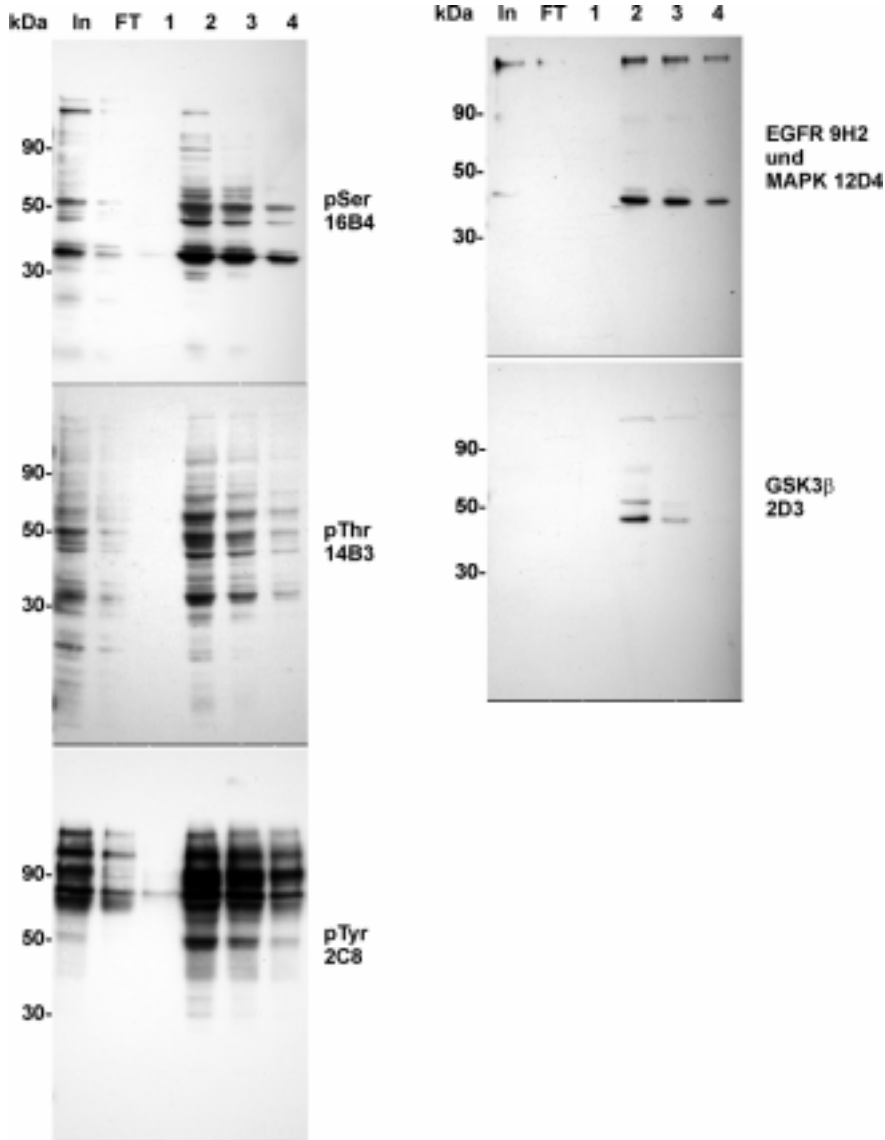
### **Reconstitution and Storage**

After use wash column with 50 ml of H<sub>2</sub>O followed by 50ml of 3% acetic acid.

Columns stored in 3% acetic acid can be reused at least once. Do not treat with NaOH!

Phosphoprotein Enrichment Kit: ALX-850-289-KI01

Phosphoproteins and –peptides can be enriched by ALX-850-289-KI01 using vanadate-treated A431 cells:



**Method: Phosphoprotein Enrichment Kit:**  
 First lane: input (unpurified)  
 second (FT) and third lane (1): flow-through  
 2-4 elution fractions from the column (enriched proteins)

**MAbs used:**

MAb to P-Ser: ALX-804-167-C100

MAb to P-Thr: ALX-804-170-C100

MAb to P-Tyr: ALX-804-171-C100

MAb to P-EGFR: ALX-804-160-C100

MAb to P-MAPK: ALX-804-061-C100

MAb to P-GSK : ALX-804-374-C100