New

# Complete separation and detection of phosphorylated proteins from eukaryotic cell lysates

The new PhosphoProtein Purification System is used for purification of phosphorylated proteins from cell lysates, significantly reducing complexity in proteomic and cell signaling studies. The affinity chromatography-based procedure enables a complete separation of phosphorylated and unphosphorylated protein fractions. Both fractions retain biological activity and can be further purified if required. PhosphoProtein Antibodies are used for highly specific immunodetection of phosphoserine and phosphothreonine residues in blotting procedures.

**The PhosphoProtein Purification Kit and PhosphoProtein Antibodies offer:**

- Complete separation of phosphorylated and unphosphorylated proteins
- Cell-signaling studies without the need for radioactivity
- A complete system, including columns, buffers, and reagents
- Detection of phosphorylated serine or threonine residues, irrespective of surrounding amino acids

**Complete separation on the basis of phosphorylation**

Post-translational modifications of individual proteins add significantly to the complexity of the proteome. Phosphorylation of serine, threonine, and tyrosine residues — which plays a vital role in cell signaling, oncogenesis, apoptosis, and immune disorders — is one of the most common post-translational modifications.

Currently available methods for chromatographic separation of phosphorylated proteins from cell lysates offer at best an enrichment of a fraction containing phosphorylated proteins, with large quantities of acidic proteins often being copurified. Until now, studies of a cell's phosphorylation status have typically used radioactivity, with its attendant handling problems, and expense.

The PhosphoProtein Purification Kit, which is based on affinity chromatography, delivers a complete separation of phosphorylated and unphosphorylated proteins from a cell lysate and therefore greatly facilitates studies of the phosphorylation status of both entire cells and specific proteins (Figures 1 and 2). The complete separation allows the ratio of phosphorylated to unphosphorylated ▶

**Highly Specific Separation of Phosphorylated Proteins**

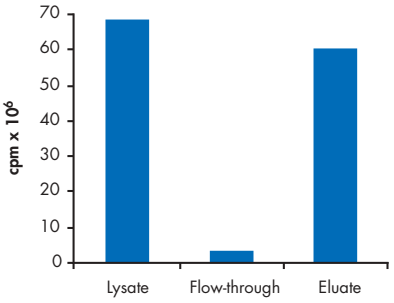


*Figure 1* *Non-stimulated Jurkat cells were radioactively labeled in vivo using $^{32}P$. Cell lysate was processed using the PhosphoProtein Purification Kit and the radioactivity in each fraction measured. (Data kindly provided by Gudrun Rehg and Sascha Dammeier, Byk Gulden, Konstanz, Germany.)*

**Complete Separation of Unphosphorylated and Phosphorylated Proteins**

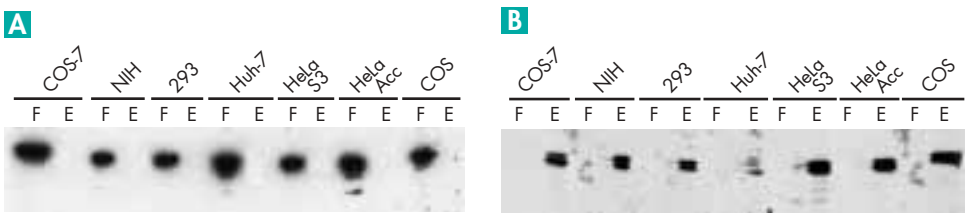


*Figure 2* *Protein-specific immunodetection of A unphosphorylated HSP-60 protein, and B phosphorylated p44 and p42 mitogen-activated protein kinase (MAPK) proteins. **F**: flow-through; **E**: eluate fractions. The antibody used to detect MAPK recognizes an epitope containing phosphorylated residues at Thr202 and Tyr204 in the p44 **(upper band)** and p42 **(lower band)** MAPK amino acid sequences. The absence of unphosphorylated HSP-60 in the eluate fraction and the absence of phosphorylated MAPK in the flow-through fraction demonstrate the complete separation of phosphorylated proteins using the PhosphoProtein Purification Kit.*

**SDS-PAGE Analysis of Flow-Through and Eluate Fractions**

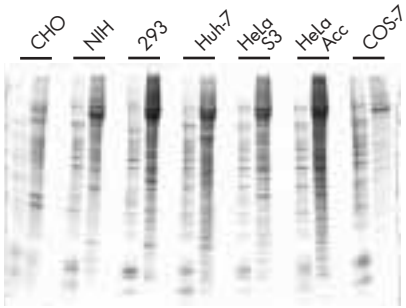


***Figure 3*** *Flow-through fractions* ***(left-hand lanes)*** *and eluates* ***(right-hand lanes)*** *from cell lysates processed using the PhosphoProtein Purification Kit. Both fractions were concentrated by a factor of 10 before loading onto the gel, and proteins were visualized by Coomassie® staining.*

forms of proteins to be easily determined. The drastic reduction of the complexity of each fraction is especially useful when studying proteins of low abundance.

### The PhosphoProtein purification procedure

Each PhosphoProtein Purification Column can be used to purify phosphorylated proteins from $10^7$ eukaryotic cells (equivalent to approximately 2.5 mg total protein). Cells are lysed in a detergent-containing buffer that provides gentle disruption of large protein complexes. Cleared lysates are loaded onto the column where phosphorylated proteins in the lysate bind to the affinity matrix, while unphosphorylated proteins are found in the flow-through fraction. After a wash step, phosphorylated proteins are eluted from the column. Depending on cell type and status, about 10% of protein loaded is recovered in the phosphorylated fraction (Table 1). The phosphorylated and unphosphorylated fractions retain full biological activity and can be further purified if desired. Nanosep® Ultrafiltration Columns are supplied with the kit to enable efficient concentration and desalting of protein fractions using a microcentrifuge.

### Highly specific detection of phosphorylated proteins

Proteins containing phosphorylated serine or threonine residues can be detected after blotting using mouse monoclonal Phospho-Serine and PhosphoThreonine Antibodies. These highly specific antibodies recognize and bind to phosphorylated serine and threonine residues, irrespective of surrounding amino acids.

The complete separation of phosphorylated proteins offered by the PhosphoProtein Purification Kit makes it unique among currently available chromatography-based methods, and an invaluable tool for research in cell signaling and proteomics. ■

**Table 1. Yields of phosphorylated proteins obtained using the PhosphoProtein Purification Kit**

| Cell type | No. of cells processed | Total protein in cell lysate | Protein loaded onto column | Protein in eluate | Phosphorylated proteins |
|---|---|---|---|---|---|
| CHO | 1.5 x $10^7$ | 3400 µg | 2500 µg | 300 µg | 12% |
| NIH/3T3 | n.d. | 2750 µg | 2500 µg | 165 µg | 7% |
| 293 | 1.5 x $10^7$ | 3650 µg | 2500 µg | 200 µg | 8% |
| COS-7 | 4.5 x $10^6$ | 1700 µg | 1700 µg | 120 µg | 7% |
| Huh-7 | 8.5 x $10^6$ | 2650 µg | 2500 µg | 235 µg | 9% |
| HT 29 | n.d. | n.d. | 2500 µg | 200 µg | 8% |
| LT 23 | n.d. | n.d. | 2500 µg | 275 µg | 11% |
| HeLa S3 | 1.8 x $10^7$ | 5950 µg | 2500 µg | 280 µg | 11% |
| HeLa Acc57 | 6.6 x $10^6$ | 2500 µg | 2500 µg | 235 µg | 9% |

*n.d.: not determined*

## Ordering Information

| Product | Contents | Cat. No. |
|---|---|---|
| PhosphoProtein Purification Kit (6) | 6 PhosphoProtein Purification Columns; buffers; reagents; 6 Nanosep Ultrafiltration Columns | 37101 |
| PhosphoThreonine Antibody Q7 (100 µg) | 100 µg anti-phosphothreonine antibody (isotype mouse IgG1, for 200 ml working solution) | 37420 |
| PhosphoSerine Antibody Q5 (100 µg) | 100 µg mixture of anti-phosphoserine antibodies (isotypes mouse IgG1 and IgM, for 200 ml working solution) | 37430 |