BD™ Phosphoprotein Enrichment Kit

A simple, affinity-based procedure for isolating phosphorylated proteins

- **Non-denaturing protocol maintains protein conformation and solubility**
- **Ideal for use with many downstream applications—mass spectrometry, 2D-PAGE, and antibody microarray analyses**
- **Increase the sensitivity of your phosphodetection experiments**

Speed and specificity are key features of the new **BD™ Phosphoprotein Enrichment Kit** from BD Biosciences Clontech. Compatible with many different analytical methods, the kit provides a straightforward, affinity-based procedure for isolating phosphorylated proteins from mammalian cells and tissues. The procedure is fast and simple, with an average cell-to-sample purification time of less than 2 hours. Each kit supplies a complete set of buffers along with six high-capacity columns for enrichment of all types of phosphoproteins, both cytosolic and membrane-bound, regardless of the amino acid modified—serine, tyrosine, or threonine.

Our enrichment procedure offers a number of advantages. As shown in Figure 1, the procedure is straightforward, consisting of four main steps: adding Extraction/Loading Buffer to the cell or tissue pellet to extract total cellular protein, loading the extract on an affinity column, washing, and finally eluting the bound phosphoprotein with a detergent-free Elution Buffer. A single buffer—Extraction/Loading Buffer—is used for both the protein extraction and affinity column steps, making buffer exchange unnecessary. This saves time and prevents sample loss. Each Phosphoprotein Affinity Column has a maximum binding capacity of approximately 4 mg of phosphorylated protein, and the procedure is non-denaturing, so phosphoproteins remain folded throughout the process, even during the extraction and elution steps.

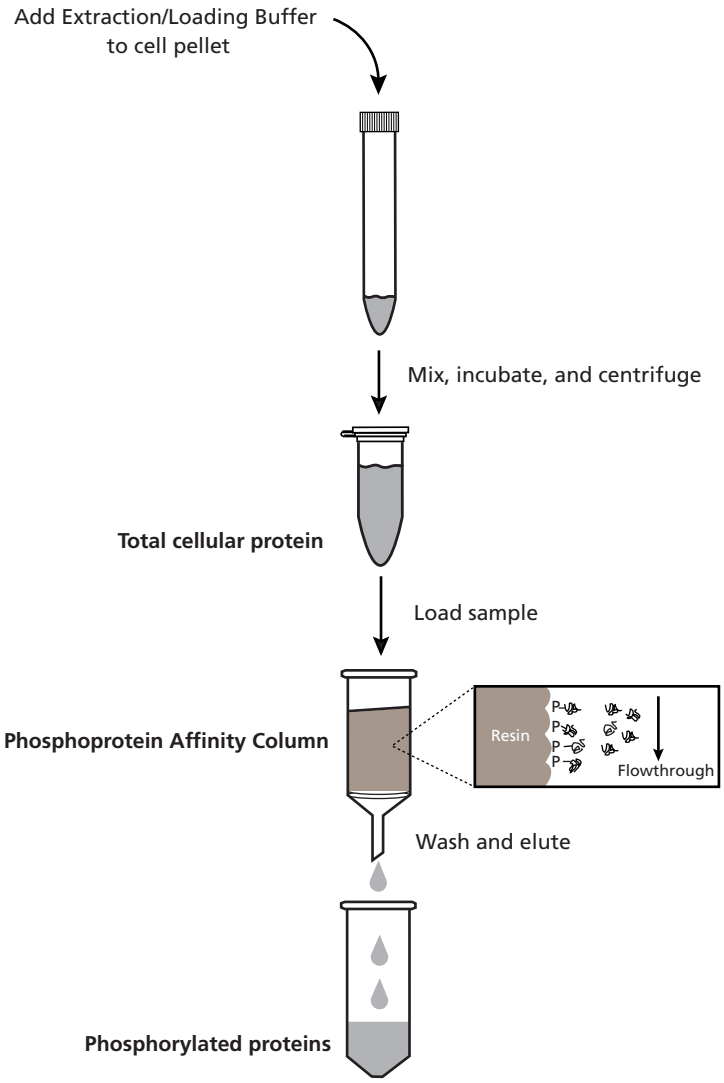

Figure 1. Overview of the Phosphoprotein Enrichment Procedure. Extraction/Loading Buffer contains a mild, non-ionic detergent for efficient, non-denaturing extraction of cellular protein.

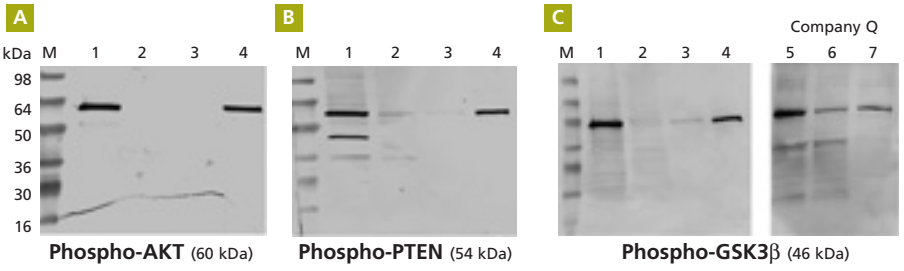

Figure 2. Highly effective enrichment of phosphorylated proteins. A Phosphoprotein Affinity Column was loaded with ~3 mg of total protein from HEK 293 cells. The extract (Lanes 1), flowthrough (Lanes 2), wash (Lanes 3), and eluate (Lanes 4) were then analyzed by Western blotting using antibodies specific for phosphorylated AKT (**Panel A**), PTEN (**Panel B**), and GSK3β (**Panel C**) proteins. The proteins were clearly detected in the eluate fraction. Compare these results to those in Lanes 5–7 (**Panel C**), which were loaded with the extract (Lane 5), flowthrough (Lane 6), and eluate (Lane 7) fractions obtained with Company Q's phosphoprotein purification system.

BD™ Phosphoprotein Enrichment Kit...continued

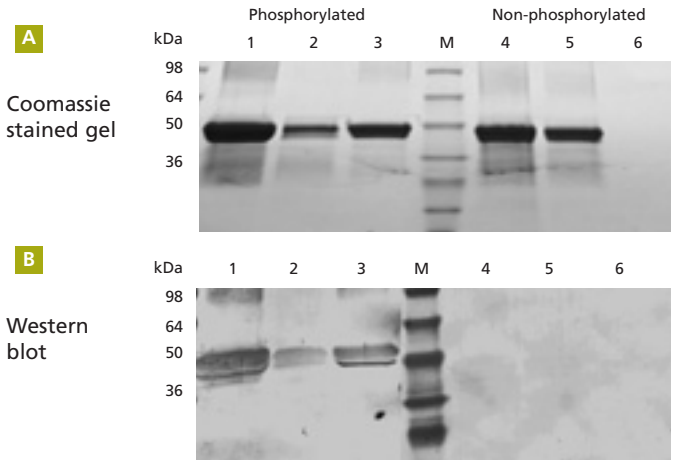

Figure 3. Phosphoprotein Affinity Columns are highly specific. Phosphoprotein Affinity Columns were loaded with either phosphorylated or non-phosphorylated (alkaline phosphatase treated) forms of ovalbumin. The resulting fractions were then analyzed by gel electrophoresis (**Panel A**) and Western blotting (**Panel B**) using a phosphoserine-specific antibody. Lanes 1–3 were loaded with the unfractionated sample, the column flowthrough, and the eluate for the phosphorylated protein, respectively. Lanes 4–6 show the same fractions for non-phosphorylated protein. The results show that the Phosphoprotein Affinity Column specifically binds only the phosphorylated form of the protein. (Compare Lanes 3 and 6.)

Why enrich for phosphoproteins?

Only a small percentage of all cellular proteins are phosphorylated at any given time (1, 2), so it is often necessary to enrich for this fraction before starting an analysis. With enrichment, you not only reduce the background but also significantly increase the sensitivity of your analysis. Rare and perhaps novel phosphoproteins are less likely to escape your detection. In the past, selective enrichment of phosphoproteins has usually involved chemical modification followed by binding to a solid support. In contrast, our method introduces no chemical changes, but relies instead on a unique resin that specifically binds the phosphate groups on native proteins. Non-phosphorylated proteins are largely eliminated during the wash step.

Highly selective enrichment of phosphoproteins

The Phosphoprotein Enrichment Kit may be used with any mammalian cell type. Cell lines tested so far include NIH 3T3, HEK 293, HeLa, Cos-7, and Jurkat. As expected, the yield of phosphoprotein varies depending on the cell line (Table I). The enrichment procedure is highly efficient as demonstrated by the Western blotting analyses shown in Figure 2. Using a colorimetric phosphate detection method, we found the majority of the phosphoprotein in the eluate; negligible traces were detected in the wash fraction. To test the specificity of the resin, we compared the eluates from columns loaded with either phosphorylated or non-phosphorylated (alkaline phosphatase-treated) ovalbumin. Only the phosphorylated form bound (Figure 3).

Phosphoprotein Affinity Columns yield a concentrated solution of phosphoprotein that can be analyzed by several different methods. Mass spectrometry and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) are two of the most common, but the enriched sample can also be analyzed by other means, including BD Clontech™ Antibody Microarrays.

Table I. Yields of phosphorylated protein obtained with the Phosphoprotein Enrichment Kit

	Protein				
				Eluate	
Cell line	Loaded (mg)	Flowthrough (mg)	Washes (mg)	mg	% of total
HEK 293	2.5	1.9	0.23	0.41	16%
Jurkat	3.3	2.4	0.30	0.52	16%
COS-7	3.1	2.4	0.26	0.47	15%
NIH 3T3	2.7	1.9	0.21	0.45	17%
HeLa	3.4	2.5	0.24	0.46	14%

The total protein in each fraction was determined with a BCA protein assay reagent kit.

Product Size	Cat. #	New Cat. #
BD Phosphoprotein Enrichment Kit❖ 6 enrichments	K1256-1	635624

❖ Coming soon. Please inquire about availability.

Components

- 6 Phosphoprotein Affinity Columns
- Extraction/Loading Buffer
- Elution Buffer
- User Manual (PT3731-1)

References

1. Alberts, B., Bray, D., Lewis, K., Raff, M., *et al.* (1994) In *Molecular Biology of the Cell, 3rd ed.*, Garland Publishing, New York, pp. 195–222.
2. Ficarro, S. B. *et al.*, (2002) *Nature Biotechnol.* **20**:301–305.

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