

# High-capacity enrichment and mass spectrometry-based characterization of phosphopeptides in complex biological samples


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## INTRODUCTION

Protein phosphorylation is one of the most abundant posttranslational modifications, which plays a key role in regulating fundamental cellular functions. Studying phosphopeptides in complex biological samples, however, presents significant challenges due to their low abundance in the proteome. Therefore, there is a need for robust and selective phosphopeptide enrichment tools. We developed the Phos-trap™ Phosphopeptide Enrichment Kit for efficient isolation of phosphopeptides from complex biological samples, such as human serum without additional pre-fractionation steps, as well as from proteolytic digests of proteins. The kit format is flexible in meeting a broad range of sample fractionation throughput requirements by performing the assay in variety of configurations (96-well plates, 8-well strips, individual vials, etc.). The assay is fully automatable and fractionated phosphopeptide samples are compatible with direct in-line or off-line detection by mass spectrometry without further processing. We demonstrate direct phosphopeptide enrichment from human serum and characterization using peptide sequencing by tandem MS analysis. Direct phosphopeptide enrichment from serum could also lead to new applications in serum biomarker research.

## Phos-trap™ Phosphopeptide Enrichment Kit

The Phos-trap™ Phosphopeptide Enrichment Kit is based on metal oxide affinity chromatography (MOAC) coupled with magnetic bead separation. Unlike the frequently used immobilized metal ion affinity chromatography (IMAC), MOAC offers significant robustness and performance advantages in phosphopeptide enrichment.



**Metal Oxide Affinity Chromatography (MOAC)**

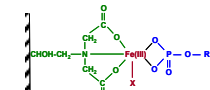
Weak Retained Acid:  $1000 < \text{pH} < 100$

Weak Retained Base:  $1000 < \text{pH} < 100$

- Based on MOAC technology
- Efficient phosphopeptide enrichment from complex biological samples such as protein digests
- Flexible and automatable kit format for 96 fractionations

- No surface pre-activation is required
- Metal oxide surfaces are stable
- Fewer non-selective interactions occur for MOAC systems
- Fractionation results are consistent and reproducible

## Immobilized Metal Ion Affinity Chromatography (IMAC)

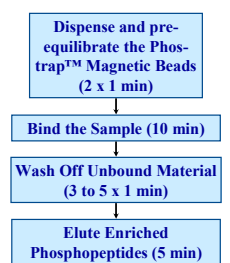


VS.

- IMAC resin needs to be pre-activated with metal ions
- Metal ions may leach during storage or fractionation
- Significant non-selective co-enrichment of highly acidic proteins is common
- Results may vary significantly among different solid surfaces

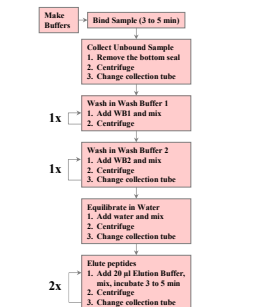
## Competitive Comparison

### Phos-trap™ kit



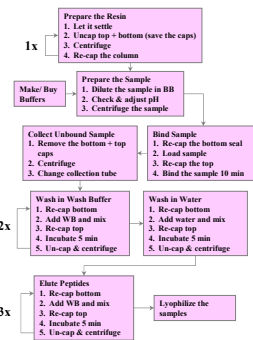
- <25 min fractionation/sample. 96 samples can be fractionated in <1 hour
- Compatible with serum samples
- Flexible and automatable kit format suitable for high throughput applications

### Competitor "P"



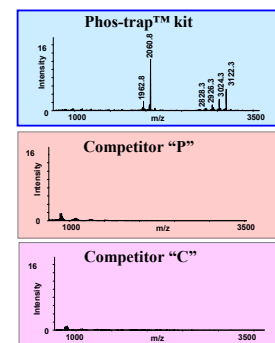
- >1 hour fractionation/sample
- Low throughput spin tube format
- Not compatible with automation
- No buffers provided
- Not compatible with serum samples

### Competitor "C"



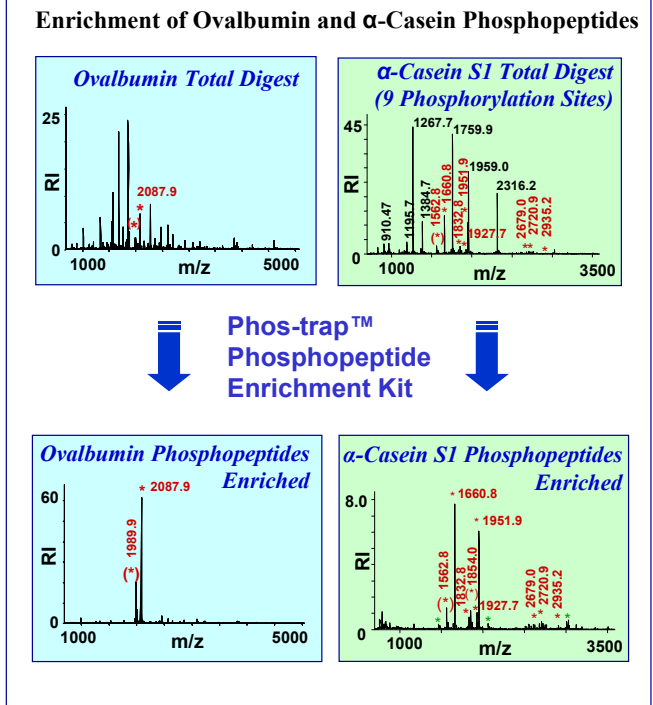
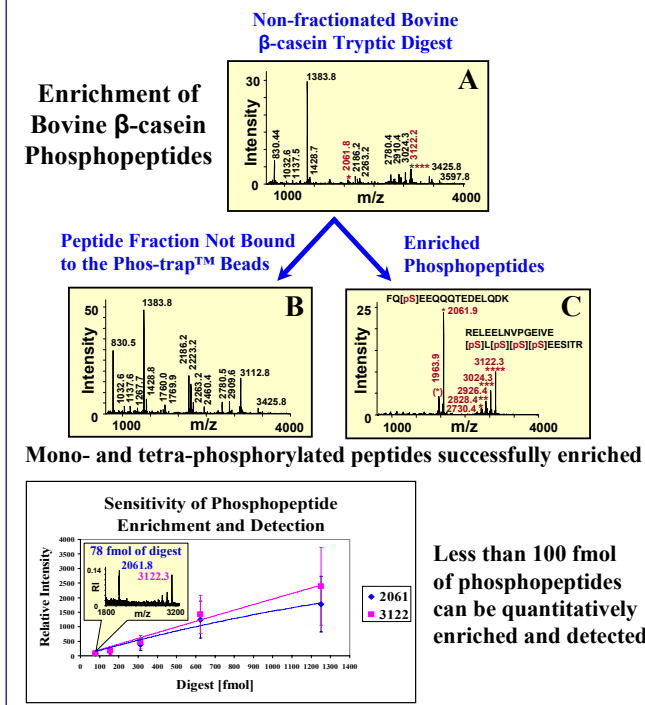
- >2 hours fractionation/sample
- Same as Competitor "P"
- Re-capping of columns and tracking the caps required.

Enriched phosphopeptides from 20 pmol of tryptic  $\beta$ -casein digest

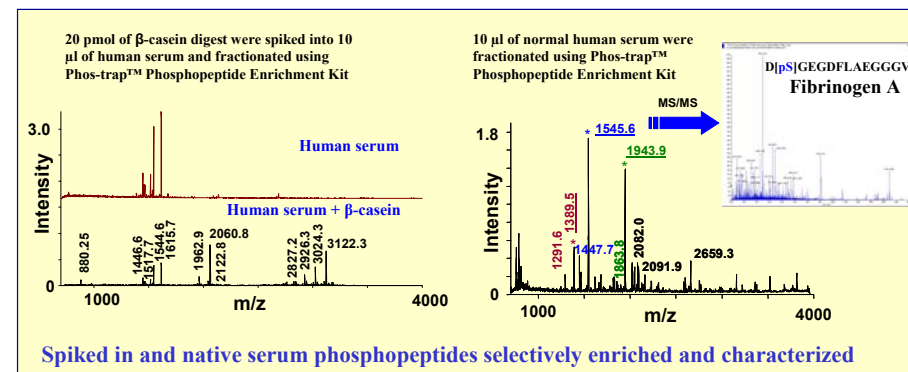


Phos-trap™ kit offers superior performance

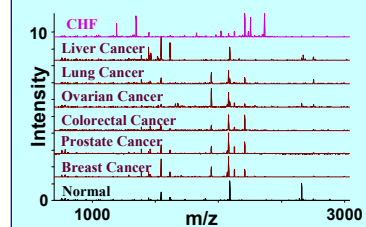
## Enrichment of Phosphopeptides From Tryptic Protein Digests



## Enrichment and Characterization of Serum Phosphopeptides



## Enrichment of Serum Biomarkers



Phos-trap™ Phosphopeptide Enrichment Kit can be used for enrichment of disease-specific serum biomarkers

## CONCLUSIONS

- Phos-trap™ Phosphopeptide Enrichment Kit can be used for efficient enrichment of phosphopeptides from protein digests and serum/plasma samples
- Flexible format meets a broad range of sensitivity and capacity requirements
- Fewer than 100 fmol of phosphopeptides can be enriched and detected from complex samples
- Assay is simple, robust, sensitive, efficient, and can be readily automated
- Potentially useful for discovery of disease-specific serum biomarkers
- Compatible with on-line or off-line analysis using MALDI TOF and other mass spectrometers