Automation of Phosphoenrichment using Magnetic Fe-NTA Beads and KingFisher™ Apex Magnetic Particle Processor

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ABSTRACT

Phosphoproteomic studies and post-translational modifications (PTMs) play a critical role in cell signaling pathways. Phosphorylation is recognized to be key factor in many human diseases both in vitro and in vivo. Presence of phosphopeptides in a sample indicates the presence of phosphorylation events, which provides a potential window into the physiology of the cell. Phosphorylation of key proteins plays a prominent role in disease progression. Several platforms are currently available for automated phosphopeptide enrichment workflows using Thermo Scientific™ Magnetic Particle Processor for high throughput applications.

RESULTS

1. Phosphoproteomics:

   a. Magnetic Particle Processor:
      - KingFisher™ MPPW-10 and the 50 µl MPPW-10 Beads were used in this study.
      - MPPW-10 Beads were washed with a phosphate buffer solution (PBS) before use.
      - Samples were incubated with MPPW-10 Beads for 30 min at RT.
      - Samples were then washed with 100 µl wash buffer.

   b. Magnetic Bead Enrichment:
      - Magnetic beads were washed for 2 min using a magnetic separator.
      - Beads were then incubated with sample for 30 min and washed again.

   c. Magnetic Bead Enrichment:
      - Magnetic beads were washed for 2 min using a magnetic separator.
      - Beads were then incubated with sample for 30 min and washed again.

   d. Magnetic Bead Enrichment:
      - Magnetic beads were washed for 2 min using a magnetic separator.
      - Beads were then incubated with sample for 30 min and washed again.

INTRODUCTION

Phosphorylation has a profound fundamental role (PF) that acts as a molecular switch in cell signaling pathways. Because phosphorylation regulates kinase to be in vivo in many human diseases, the development of robust and sensitive phosphoprotein enrichment workflow is crucial. Several platforms are currently available for the enrichment of phosphopeptides, enabling the identification of phosphorylation events in complex samples. These platforms include magnetic bead-based methods, affinity chromatography, and liquid chromatography.

MATERIALS AND METHODS

Sample Preparation:

- Samples were collected from HeLa S3 cells and subjected to control and treatment conditions.
- Samples were prepared using a KingFisher™ MPPW-10 and 50 µl MPPW-10 Beads.
- Samples were incubated with MPPW-10 Beads for 30 min at RT.
- Samples were then washed with 100 µl wash buffer.

Phosphopeptide Enrichment:

- Magnetic beads were washed for 2 min using a magnetic separator.
- Beads were then incubated with sample for 30 min and washed again.

CONCLUSIONS

Phosphoproteomic studies and post-translational modifications (PTMs) play a critical role in cell signaling pathways. Phosphorylation is recognized to be key factor in many human diseases both in vitro and in vivo. Presence of phosphopeptides in a sample indicates the presence of phosphorylation events, which provides a potential window into the physiology of the cell. Phosphorylation of key proteins plays a prominent role in disease progression. Several platforms are currently available for automated phosphopeptide enrichment workflows using Thermo Scientific™ Magnetic Particle Processor for high throughput applications.