Agilent Phosphochip

A New HPLC-Chip for Phosphopeptide Analysis
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Agilent Technologies

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HPLC-Chip/MS
For all Agilent 6000 Series Mass Spectrometers
HPLC-Chip/MS

Features

Performance enhanced
• Zero dead volume for better chromatographic performance
• LC/MS sensitivity

Fully integrated design
• Laser-ablated channels
• Analytical column
• Enrichment column
• Micro-valve connection
• Nano-electrospray tip
• Micro-filters

Robustness & ease-of-use
• No clogging of spray needle
• Plug-&-Play replacement
HPLC-Chip
Automated workflow

- Chip-Cube
- Stator
- Rotor

- Micro-valve(s)
- Nanoflow pump

- Waste
- Autosampler

- Sample loading
  - Mostly aqueous with C18
  - µL/min - flow

- Sample analysis
  - Organic gradient
  - nL/min - flow

Our measure is your success.
Ease-of-Use & Flexibility

Plug-&-Play in 3 steps…
1. Insert chip
2. Click
3. Switch-on pumps

…ultimately yields flexibility!
For instance

• Protein ID Chip/QTOF for high accurate mass peptide sequencing
• Simple chip transferred to QQQ for high sensitivity peptide quantification
• Swift switch between different chip types and/or chemistries allows for instantaneous use of the QQQ for small molecule chip-applications
Challenges in Phosphoproteome Analysis

Low abundance of phosphoproteins in cells
• <3% of all proteins

Low stoichiometry of phosphorylation
• Transient event
• <20% phosphorylated

Enrichment of phosphopeptides necessary for identification

Inconsistent trapping methods
• IMAC: Ga$^{3+}$
• Metal Oxide: TiO$_2$
• Phosphotyrosine specific antibody for pY-containing peptides
Phosphochip Description

Packing material used:
RP1: ZORBAX Extend 5 µm, 300Å
TiO₂: 10 µm spheres
RP2: ZORBAX Extend 5 µm, 300Å
AC: Reprosil-Pur C18-AQ 5 µm
Three Modes of Analysis

Standard peptide analysis with back flushing

Phosphopeptide analysis only with organic plug and forward flushing with phosphopeptide elution buffer and gradient

Combined peptide and phosphopeptide analysis using gradient, forward flushing with phosphopeptide elution buffer and gradient again
Chip Validation

No enrichment

Enrichment on TiO₂
Retention Time Reproducibility

% RSD 0.12

% RSD 0.19
Example of Erk1 Phosphopeptide Identified
Phosphochip

Phosphochip Features

- Completely integrated microfluidic design
- Simplified enrichment/analysis
- Increased sensitivity for phosphoproteome analysis
- Faster time-to-result
- More routine PTM analysis

Phosphochip Workflow

- Sandwiched RP-TiO$_2$-RP trapping column for phosphopeptide enrichment
- Dual modes of analysis for both phosphorylated and non-phosphorylated peptides from complex protein digest