Integrated Protein Digestion HPLC

Perfinity iDP

PERFINITY
Perfinity iDP, a new platform for protein analysis, automates the protein digestion process

The Perfinity iDP (Integrated Digestion Platform) automates the protein analysis workflow from protein digestion to HPLC separation and MS detection, significantly reducing sample preparation times and enhancing reproducibility.

**Manual Method**

- Protein digestion 18-24 hours
- Solid phase extraction
- Re-suspension
- HPLC or LC/MS analysis

**Perfinity iDP**

- Protein digestion (1-4 minutes)
- Desalting
- HPLC or LC/MS analysis

Perfinity iDP reduces the entire sample preparation workflow down to **20 minutes**

**Reduce trypsin digestion time to 1-4 minutes**

**Automated Digestion Workflow**

**Digestion**
Proteins are rapidly digested by trypsin immobilized onto the stationary phase of the column.

**Desalting**
Tryptic peptides are concentrated and desalted on the desalting column.

The protein sample is delivered into the trypsin column by optimized digestion buffer. Sample can be multi-plexed using two desalting column to increase throughput.

Mobile phase

**Trypsin column**

**Desalting column 1**

**HPLC or LC/MS analysis**

Reverse phase column

Detector
Features

- High-efficiency trypsin column provides fast on-line trypsin digestion in 1-4 minutes
- Perfinity iDP Software provides a user-friendly interface
- Full automation enables reproducible results
- Coupling with LC/MS products opens a wide range of protein applications
Fast On-Line Trypsin Digestion

1-4 minutes trypsin digestion using a high-efficiency trypsin column

The benefits of using Perfinity iDP with the immobilized trypsin column are:

1. Fast trypsin digestion in 1 to 4 minutes
2. Continuous sample analysis on reusable trypsin column
3. Reduced chymotrypsin activity and deamidation

Insulin was digested within 1 minute using the trypsin column as shown in the figure at right.
* Optimal digestion time depends on proteins.

Optimized Perfinity iDP digestion buffer

The optimized trypsin digestion buffer in combination with the trypsin column enables highly efficient digestion.

The figure to the right shows a comparison between the optimized digestion buffer and the commonly used tris buffer. The optimized digestion buffer provided a higher peptide recovery rate with a short digestion time.

Increased throughput by parallel sample processing with two desalting columns

Two desalting columns allow parallel processing of samples. One desalting column is used to trap digested peptides, while peptides from the previously injected sample are eluted from the other desalting column. This parallel sample processing allows analysis of up to 200 samples/day.

The figure to the right is an example of parallel sample processing, which increases throughput while maintaining reproducibility.
Easy Operation by Perfinity iDP Software

The Perfinity iDP software supports the total workflow from method development to analysis of samples.

**Method settings**
After selecting a method, users can quickly create and optimize methods by entering a few parameters, such as digestion time, column dimensions, and gradient time. Perfinity iDP methods are then automatically generated for running samples.

**Batch file creation**
Batch files for sample analysis are quickly generated. Users enter minimal information such as methods, data file names, and injection volumes.

**Seamless connection with LabSolutions**
Analysis results can be viewed in the LabSolutions post-run window, which can be accessed through the Perfinity iDP software.
Full Automation Enables Reliable Analysis

Increased reproducibility by automation of workflow

Perfinity iDP minimizes human error by automating all steps from digestion to desalting to LC/MS analysis. The figure at right shows reproducible chromatograms of reduced and alkylated transferrin analysis.

Reproducibility of 20 peptides that was produced by digestion of reductive alkylated transferrin

Minimized carryover

Reliable instrumentation and optimized methods result in very low levels of carryover. The figure at right shows a blank injection after a transferrin injection to illustrate minimized carryover with reliable results using Perfinity iDP.
**Wide application range with LC/MS**

**Application: Detection of IgG digested peptides by LCMS-2020**

IgG was analyzed Perfinity iDP with LCMS-2020. Main peptide fragments were easily confirmed after 4 minutes digestion.

![LC chromatogram (UV 214 nm)](image)

![MS (TIC) chromatogram of IgG (300 pmol)](image)

- **MS spectrum of peak 1 (retention time: 10.01 min)**
- **MS spectrum of peak 2 (retention time: 17.30 min)**
- **MS spectrum of peak 3 (retention time: 30.25 min)**
System configuration

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Columns and Consumables

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