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Perfinity Immobilized Lys-C Column 2.1x33mm

(Product Numbers: 100-1021-LysC, 100-1021-LysC-P)

Recent successes in proteomics are based on the fact that proteins are reduced to more easily identifiable fragments by cleavage with proteolytic enzymes, the most popular being trypsin. However, trypsin is prone to multiple shortcomings including the inability to cleave arginine or lysine residues adjacent to prolines and incomplete or poor digestion of tightly folded proteins. One way to bypass these shortcomings is to use additional enzymes, such as the endoproteinase Lys-C. Similar to trypsin, Lys-C cleaves the carboxyl side of lysine but has the added benefit of being able to cleave adjacent to prolines.

Typical Lys-C digestion is performed at substrate:enzyme mass ratios of 20:1 to 100:1 for 2 to 18 hours at 37°C. Similar to other proteolytic enzymes, increased concentrations may decrease digestion times but increases the likelihood of self-digestion. Immobilization of Lys-C, demonstrated the ability to dramatically reduce digestion times while also reducing self-digestion.

Materials recommended but not provided

- A Perfinity Workstation or integrated Digestion Platform. Multicolumn systems that greatly facilitate automation.
- 50mM Tris buffer for use during digestion
- Perfinity Optimized Re-equilibration Buffer (optimal clean-up)

I. Protein Digestion Procedure -

Equilibrate the column by washing with 20 column volumes of 50mM Tris buffer. If applicable, set the column heater to 37-40°C and allow for temperature equilibration. Set the loading pump to the desired digest flow rate and inject the sample. Either collect the sample or transfer to a desalting column. After desalting at initial mobile phase conditions bring desalting cartridge in line with RPC column for analysis. Between sample injections, wash the column with at least 20 column volumes (2mL/min. for 1 min.) of Perfinity Optimized Re-equilibration Buffer followed by a 20 column volume re-equilibration with 50mM Tris buffer. Purge column with re-equilibration buffer followed by 50mM Tris buffer if column has been sitting for an extended period of time as idle Lys-C is prone to auto-digestion and might result in aberrant peaks during blank runs. Follow this protocol anytime aberrant peaks are detected during blank runs.

II. **Operational Specifications** –

Flow Rate Range	10-500 uL/min. 50 uL/min. recommended
Pressure Max	170 bar/2500 psi
pH	8 (optimal)
Digestion buffer	50mM Tris buffer (TB)
Operating Temperature (°C)	25-50; 37-40 is optimal (>40 reduces column life)
Storage Temperature (°C)	4°C for extended period of time (refrigerate in 50mM TB)

1. To maintain digestion efficiency, cationic salts such as $Ca2^{+}$ and Na^{+}, should be omitted or reduced from samples.

III. **Operational Parameters** –

Operating Flow Rate (uL/min.)	10	25	50	100	500
Reaction Time (min.)	10	4	2	1	0.2

IV. **Optimization** –

All proteins vary with regards to digestion; adjust temperature & flow rate accordingly

To increase digestion: increase temperature, decrease flow rate, reduce & alkylate protein
To decrease digestion: decrease temperature and increase flow rate

-Reduction/Alkylation Procedures:

Add 40 molar excess reducing agent (e.g. DTT, or BME) in 3 M urea and incubate for 15 min. at 50°C. Add 40 molar excess alkylating agent (e.g. 4-vinyl pyridine, iodoacetamide, or iodoacetic acid) and incubate for 15 min. at room temperature. Add 40 molar excess cysteine to prevent alkylation of primary amines.

V. **Column Storage** –

Store column at 4°C in 50mM Tris buffer with end plugs in place to prevent drying. For long term storage, store the column in Tris buffer with 0.02% sodium azide as a preservative.

VI. Test for enzymatic activity

- 400 ug/mL HuIgG in Tris
- 50mM Tris buffer

Set your UV Detector to monitor absorbance at 214 and 280 nm, and equilibrate the column using Tris buffer.

Human IgG was reduced using Perfinity SMART Immobilized TCEP Resin for 45 minutes prior to online digestion via Perfinity Immobilized Lys-C Column. Reduced HuIgG (20ug) was digested online for 5 minutes, followed by peptide analysis using a C18 Reverse-Phase Column and 60 minute gradient.

Figure 1: Comparison of Offline (18hr, 37 °C) and Online Lys-C Digestion of Human IgG at 280nm using the Perfinity Workstation.

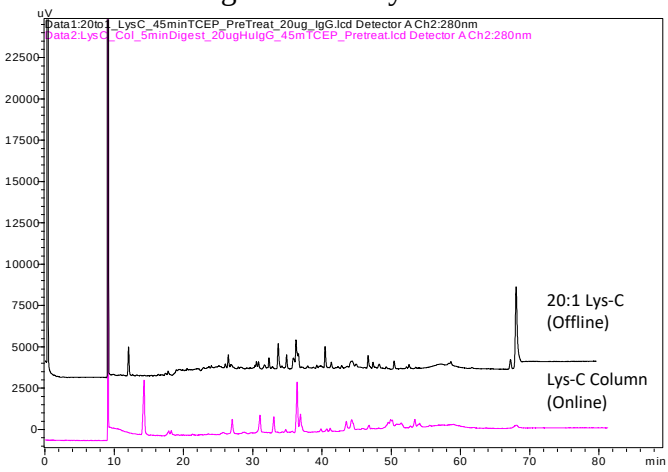
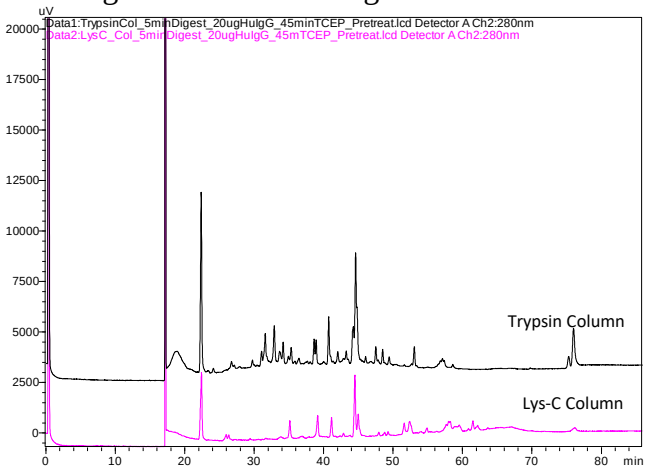


Figure 2: Comparison of Perfinity Immobilized Lys-C and Immobilized Trypsin Column Digestions of Human IgG at 280nm.



Digestion Column:	Perfinity Immobilized Enzyme Column (2.1 x 33 mm)
Analysis Column:	Phenomenex C18 (2.1 x 150 mm)
Column Oven Temp:	40°C
Injection Volume:	50 µL
Digestion Buffer:	50mM Tris Buffer
Mobile Phase A:	98% H_2O, 2% Acetonitrile, 0.1% TFA
Mobile Phase B:	10% H_2O, 90% Acetonitrile, 0.1% TFA
Analysis Method:	60 minute gradient from 2 to 50% Mobile Phase B