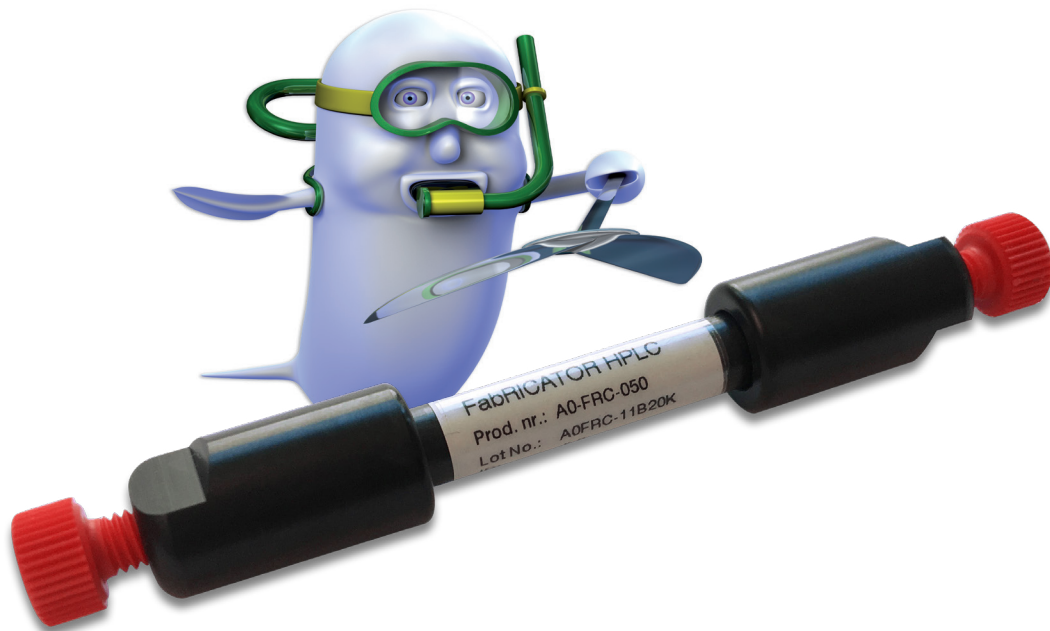


# FabRICATOR<sup>®</sup>-HPLC

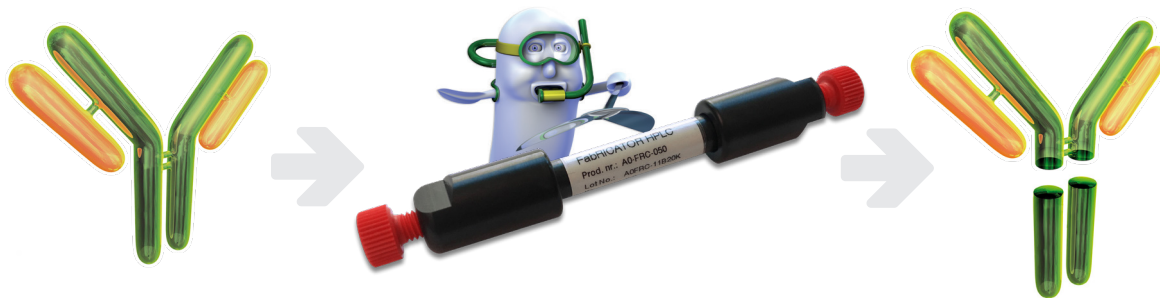


Automated Antibody Subunit Generation

SmartEnzymes™



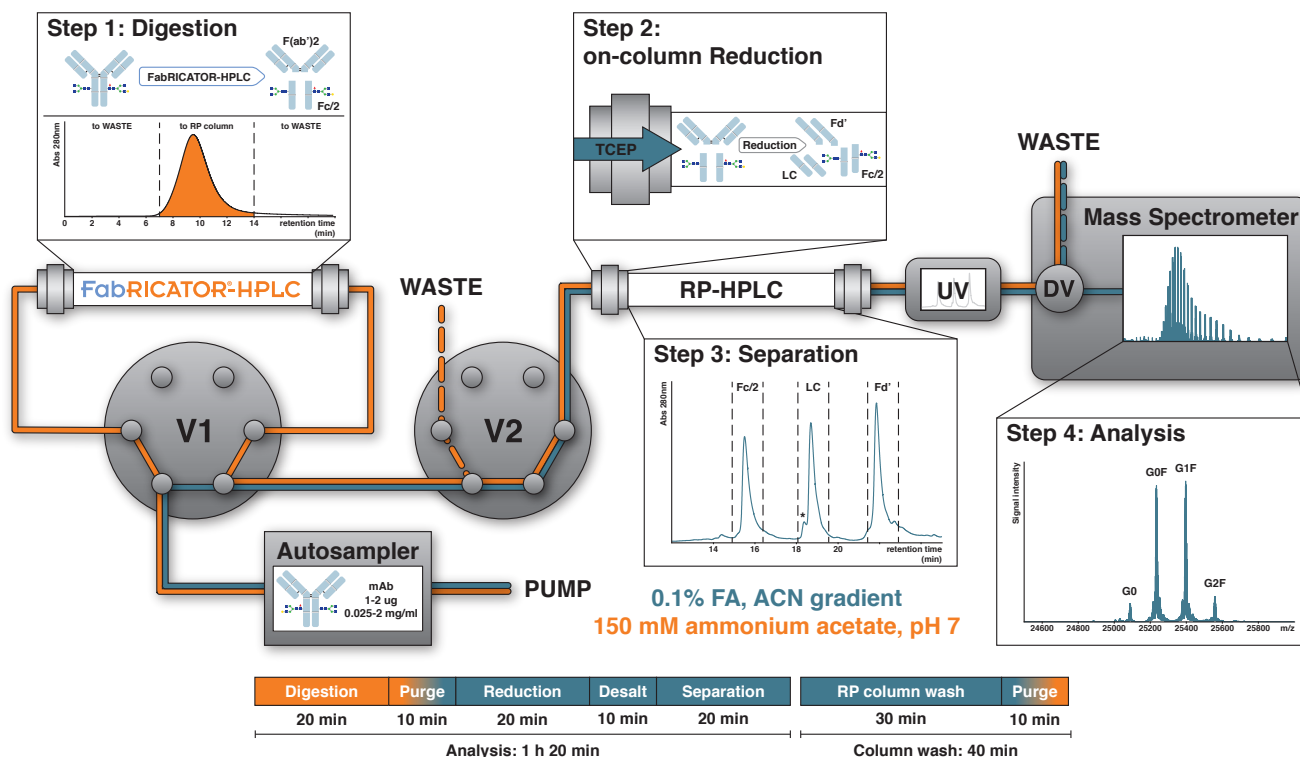
# FabRICATOR<sup>®</sup>-HPLC



FabRICATOR-HPLC offers on-column digestion of monoclonal antibodies for rapid subunit generation in automated middle-level workflows.

FabRICATOR-HPLC contains the FabRICATOR (IdeS) enzyme immobilized on an HPLC compatible resin, generating F(ab')<sub>2</sub> and Fc fragments without risk of over-digestion. In-house testing (Fig. 2 and 3) highlights the stability and reproducibility with the column delivering consistent digestion for 14 days at 37°C. More than 450 injections of mAb were made during testing and no carry-over was observed.

FabRICATOR-HPLC can be used in a standard LC-MS setup for routine analysis (Fig. 1). But more advanced configurations are possible, for example with 2D-LC. Ultimately, a bioreactor can be connected directly to the MS in an automated online middle-level workflow. This would significantly reduce operator time, sample handling errors and increase throughput.



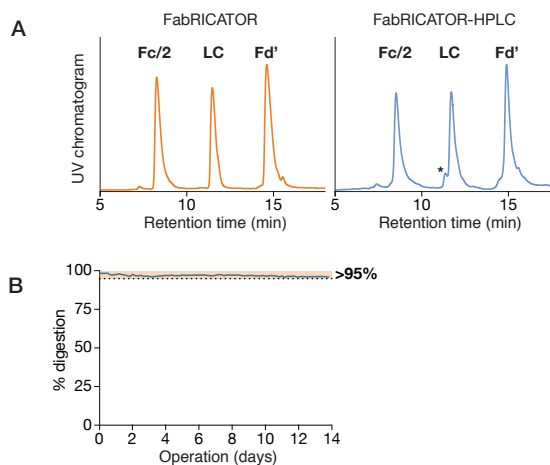
**Figure 1.** Potential set-up for an automated middle-level workflow using FabRICATOR-HPLC. FabRICATOR digests IgGs to F(ab')<sub>2</sub> and Fc, which is well suited for high resolution MS analysis.

## Robust On-column Performance

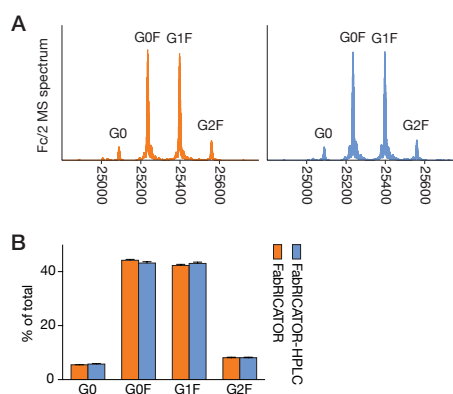
Digestion efficiency is maintained by FabRICATOR-HPLC during continuous operation at 37°C for up to 14 days. In our tests, 2 µg trastuzumab samples were injected every 4h under native conditions in 150 mM ammonium acetate, pH 7 at a flow rate of 25 µl/min. The resulting antibody fragments were reduced on-column and analyzed using the automated subunit analysis workflow described in Fig. 1 (Fig. 2a). More than 95% of the antibody was digested during the entire 14-day period. (Fig. 2b).

## Reproducible Glycan Analysis

The performance and operational stability of the FabRICATOR-HPLC column are demonstrated here using analysis of trastuzumab Fc glycosylation as an example. Automated middle-level analysis using FabRICATOR-HPLC yielded mass spectra virtually indistinguishable from the one obtained from a standard in-solution FabRICATOR digestion workflow (Fig. 3a). The resulting Fc glycosylation profiles were stable and reproducible during 14 days of continuous operation with standard deviations of less than 0.5% for all glycoforms (Fig. 3b).



**Figure 2. a)** Comparison between digestion of trastuzumab using standard in-solution FabRICATOR protocol (left) and digestion using the automated FabRICATOR-HPLC workflow (right). The asterisk marks LC fragments that are not completely reduced with one intramolecular disulfide bridge intact. **b)** Quantification of digestion performance over a period of 14 days.



**Figure 3. a)** Deconvoluted mass spectra of the trastuzumab Fc/2 fragment from in-solution FabRICATOR digestion (orange), or FabRICATOR-HPLC (blue). **b)** Glycosylation profiles of trastuzumab generated by in-solution FabRICATOR digestion (orange, n=10) or FabRICATOR-HPLC (blue, n=28, 2 samples per day).

### Product Overview

Column hardware: PEEK/biocompatible  
 Column dimensions: 2.1 mmD x 50 mmL  
 Support resin: POROS® (see Legal and Disclaimers, p. 39)  
 Typical flow rate: 0.025-0.05 mL/min  
 Maximum Pressure: 100 bar

Operating pH: 6.5 - 8.0  
 Operating temperature: 37°C  
 Storage conditions: +4-8°C (Do not freeze!)  
 Number of days of continuous operation: >10\*  
 Injections per column: >200\*  
 Start material: Human IgG1-4, Fc-fusion proteins

\*Depending on specific application

# FabRICATOR®-HPLC

FabRICATOR-HPLC contains the FabRICATOR enzyme immobilized in an HPLC column for fast on-column digestion of monoclonal antibodies.




Product ID	Description	EUR	USD
A0-FRC-050	FabRICATOR-HPLC	1,599	1,799


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