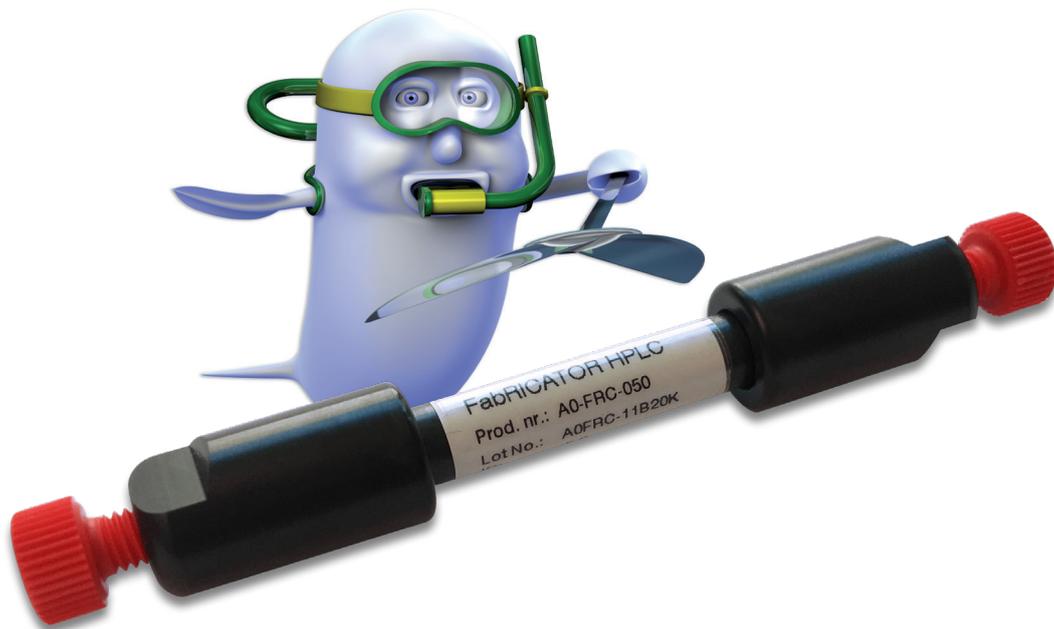




FabRICATOR[®]-HPLC



Automated Antibody Subunit Generation

SmartEnzymes™



FabRICATOR®-HPLC

FabRICATOR®-HPLC



FabRICATOR-HPLC contains FabRICATOR (IdeS) enzyme immobilized on an HPLC compatible resin, generating F(ab')₂ and Fc fragments without risk of over-digestion. In-house testing (see page 3 for details) 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. But more advanced configurations are possible, for example with 2D-LC. Ultimately a bioreactor can be connected directly to the MS in an auto-

mated online middle-level workflow (Fig. 1). This would significantly reduce operator time, sample handling errors and increase throughput.

Key Characteristics

- ▶ FabRICATOR-HPLC column enables automated subunit generation
- ▶ Complete digestion of 10 µg IgG in less than 5 minutes
- ▶ No carry-over observed in testing
- ▶ Reduce manual sample handling errors
- ▶ Increase throughput of subunit analysis

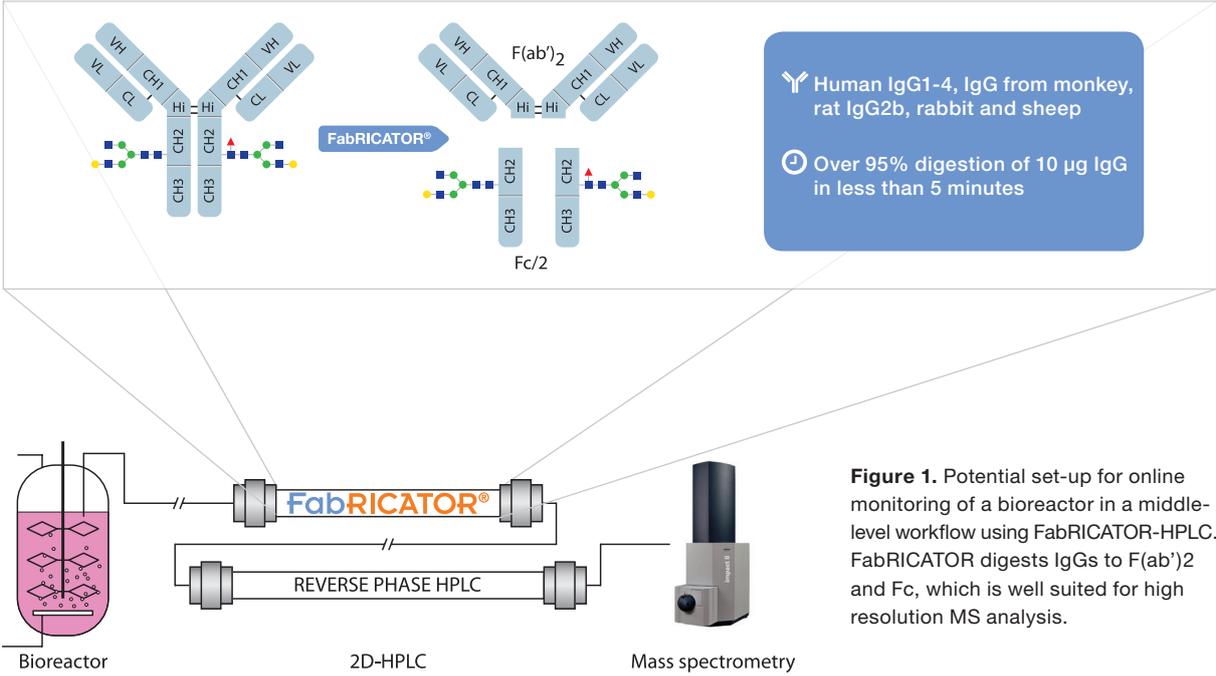


Figure 1. Potential set-up for online monitoring of a bioreactor in a middle-level workflow using FabRICATOR-HPLC. FabRICATOR digests IgGs to F(ab')₂ and Fc, which is well suited for high resolution MS analysis.

Robust On-column Performance

Digestion efficiency is maintained by FabRICATOR-HPLC during continuous operation at 37°C for 14 days. In our tests, 10 µg trastuzumab samples were injected every 40 minutes under native conditions in PBS at a flow rate of 25 µl/min. The resulting antibody fragments were collected, reduced and analyzed by RP-HPLC (Fig. 2a). Digestion of the antibody was complete until around day 10, where some residual intact heavy chain became detectable. However, even in the samples from day 14, digestion exceeded 90% (Fig. 2b).

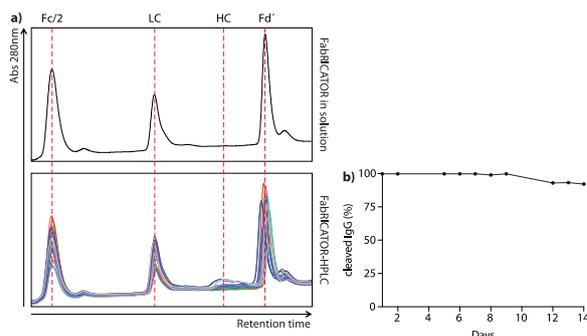


Figure 2. a) Comparison between digestion of trastuzumab using standard in-solution FabRICATOR protocol (top) and digestion using FabRICATOR-HPLC (bottom). UV traces from 20 samples taken on 10 different days over 14 days overlaid. **b)** Quantification of digestion performance over a period of 14 days.

Reproducible Glycan Analysis

The performance and operational stability of the FabRICATOR-HPLC column is demonstrated here with trastuzumab Fc glycosylation. Ten samples taken on different days over a 14-day period of continuous operation were analyzed by LC-MS (Fig. 3a). The resulting glycosylation profiles were very reproducible with standard deviations of less than one percent. Comparison between samples digested by FabRICATOR-HPLC with samples prepared with the standard in-solution FabRICATOR protocol showed no significant differences in the glycosylation profiles (Fig. 3b).

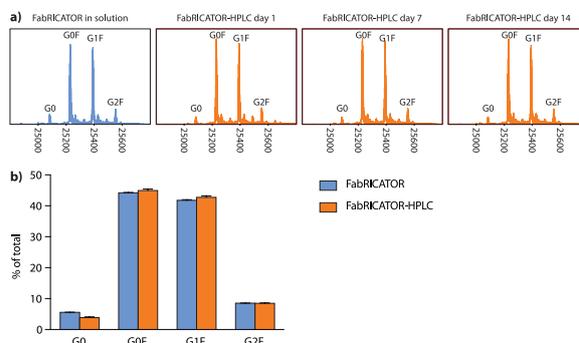


Figure 3. a) Deconvoluted mass spectra of the trastuzumab Fc/2 fragment from in-solution FabRICATOR digestion (black), or FabRICATOR-HPLC (orange) on days 1, 7 and 14. **b)** Glycosylation profiles of trastuzumab generated by in-solution FabRICATOR digestion (black, n=6) or FabRICATOR-HPLC (orange, n=20).

Product Overview

Column dimensions: 2.1 mmD x 50 mmL

Support resin: POROS™

Typical flow rate: 0.025-0.05 mL/min

Maximum Pressure: 100 bar

Operating pH: 6.5 - 8.0

* Depending on specific application

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

FabRICATOR®-HPLC



Product ID	Description	EUR	USD
A0-FRC-050	FabRICATOR-HPLC	1,750	1,980

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