

Automating Titer Measurement

Tech Note

Automating At-Line Titer Measurement with a Novel Fit-For-Purpose Chromatographic Device

Authors: Jaime Bravo, Connie Yuan, and Craig Love at Rohnert Park, CA

Abstract

A number of process analytic technologies (PAT) have been developed for optimizing cell attributes and culture media components, but increasing viable cell density (VCD) does not always produce a commensurate increase in product titer¹. Therefore, the measurement of antibody titers in CHO cell cultures is a critical process parameter (CPP) for optimizing cell culture conditions. Titer measurement must be rapid, robust, and the assay must have a wide dynamic range to monitor product yields throughout a bioreactor run.

Here, we describe a novel, compact device, the Tridex Protein Analyzer, capable of rapid, automated at-line measurement of antibody titers from stirred bioreactors using an aseptic sampling device. Assay linearity and accuracy were evaluated over a measurement range of 0.1 to 6.5 g/L using a simulated cell culture titer curve spiked with bovine serum albumin (BSA) and human IgG. Manual and automated aseptic sampling measurements using the Tridex analyzer compared very favorably with a commercial Protein A HPLC system. Excellent intra-assay precision (CV < 3%) was demonstrated for both off-line and at-line measurements with the Tridex analyzer. Measurement results were also unaffected by BSA levels up to 2.5% (v/v).

1. *Appl Microbiol Biotechnol* (2015) 99:4645–4657.

Simulated Titer Curve

- › Samples Spiked with BSA and Purified HulgG to Simulate an 18-day Bioreactor Campaign

Day	IgG (g/L)	% BSA
2	0.10	0.10
4	0.20	0.15
6	0.75	0.23
8	1.75	0.34
10	3.00	0.51
12	4.75	0.76
14	5.75	1.14
16	6.25	1.71
18	6.50	2.56



Compact Footprint



Automated At-Line Titer Measurement



Correlates to Off-Line HPLC



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Analytical Conditions

Instrumentation

- › Agilent 1100 HPLC system with autosampler and 2.1 x 30 mm Protein A column for off-line titer measurement.
- › Flownamics Seg-Flow® 1200 with F-Series FISP probe for automated aseptic at-line sampling with Tridex analyzer.
- › IDEX Health & Science Tridex Protein Analyzer with proprietary titer module for manual and automated at-line analyses.

Real Time Antibody Titer

› The Tridex analyzer is a self contained system with pumps, valves, analytical module, detector, and reagent pack, built into a small footprint:

Height: 21" (53.3 cm)
 Width: 10" (25.4 cm)
 Depth: 17" (43.2 cm)
 Weight: 37 pounds (17 kg)*
 *not including full reagent pack

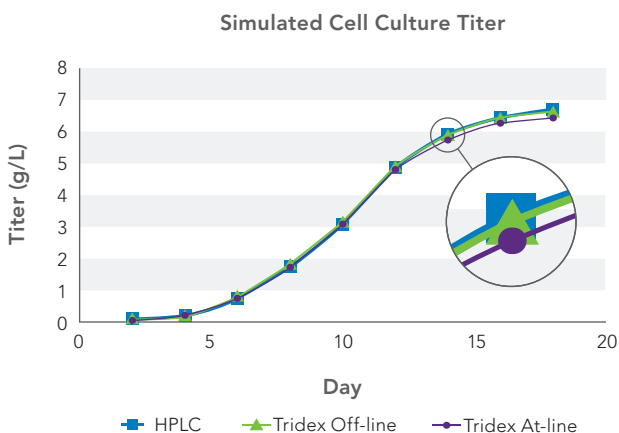
- › Measurement range from 0.1 to 10 g/L without sample dilution or recalibration.
- › Titer results are automatically displayed on integrated monitor in under 5 minutes.
- › Reagent pack and analytical module provide up to 1,000 analyses.



Evaluation Results

Comparing At-Line and Off-Line Titer Measurements with Off-Line Protein A HPLC

- › Antibody titer at each level was calculated using the average of three measurements for all data sets.
- › Accurate at-line and off-line titer measurements up to 6.5 g/L were obtained using the Tridex analyzer without sample dilution using linear calibration curves.



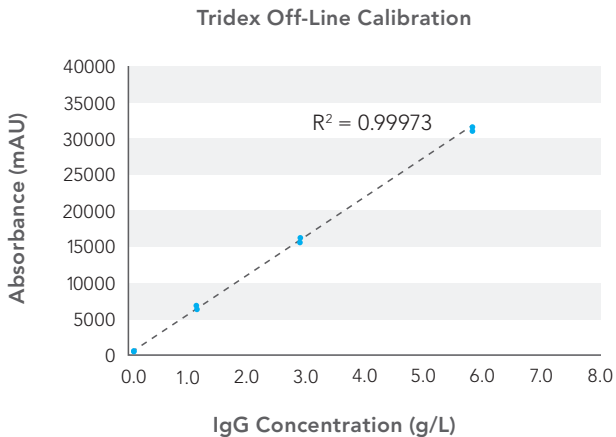
Accuracy Table

- › Tridex measurement errors calculated relative to Protein A HPLC titer values.
- › Relative errors < 10% were obtained for Tridex off-line and at-line titer values across the measurement range.
- › Excellent intra-assay precision (CV < 3%) demonstrated for both off-line and at-line measurements.

Day	ProA HPLC Titer (g/L)	Tridex Off-Line Error	Tridex At-Line Error
2	0.10	1.99%	9.21%
4	0.21	2.79%	3.08%
6	0.76	7.31%	4.31%
8	1.75	5.90%	1.54%
10	3.09	2.70%	1.30%
12	4.86	1.86%	0.62%
14	5.92	0.21%	2.62%
16	6.44	0.37%	2.41%
18	6.68	0.56%	3.74%

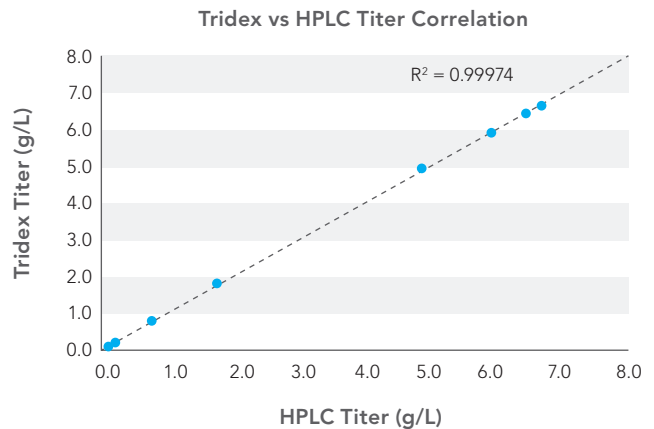
Calibration Curves Generated Using a Human IgG Isotype Control Standard

- › Standard measurements at 0.1, 1.0, 2.5, and 5.0 g/L used for generation of Tridex and HPLC calibration curves.
- › Excellent fit (R^2) of linear regression across calibration range.
- › Extended dynamic range allows extrapolation to higher titer values.



Correlation of Tridex and Protein A HPLC Titer Measurements

- › Plot of Tridex vs. HPLC titer values shows excellent agreement across titer range.
- › Measurement results unaffected by changing BSA levels up to 2.5% (v/v).
- › Demonstrates successful application of a fit-for-purpose device for antibody titer.



Summary

- › A novel fit-for-purpose chromatographic device was successfully integrated with a Flownamics Seg-Flow unit for automated at-line titer measurement of a simulated cell culture.
- › Linear response of the Tridex Protein Analyzer was demonstrated from 0.1 to 6.5 g/L without recalibration or sample dilution.
- › Automated at-line and off-line titer measurements from the Tridex analyzer were within 10% of the titer values from off-line Protein A HPLC.
- › Excellent intra-assay precision ($CV < 3\%$) was obtained for both off-line and at-line measurements using the Tridex analyzer.
- › Titer results were obtained in under 5 minutes for off-line measurements, and under 10 minutes for automated at-line measurements using the Tridex analyzer.



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