



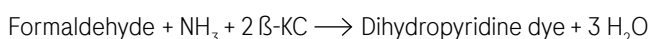
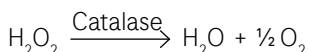
Methanol Assay for Cedex[®] Bio & Bio HT Analyzers

Reliable and convenient determination in process control

The Methanol assay is developed for automated testing on Cedex Bio and Cedex Bio HT Analyzers, and it provides fast and accurate determination of methanol (methyl alcohol) in samples of fermentation media and other aqueous solutions. The assay is highly specific for methanol, even with other alcohols in a sample background.

Assay principle

The assay uses an alcohol oxidase enzyme (AOX) for oxidation of methanol to formaldehyde. Hydrogen peroxide released in this reaction is immediately degraded to water by catalase to avoid further interference. The formaldehyde reacts with ammonia and a β -keto carbonyl compound (β -KC), forming a Hantzsch dihydropyridine dye which is highly specific for formaldehyde. The generated dye is photometrically determined by its absorption at 378 nm, relating to the methanol concentration of the sample.



Perfect for bio-process control

- Fully automated assay
- No sample pretreatment required
- Ready-to-use reagents
- Long on-board and calibration curve stability

Typical Applications

1. Recombinant protein expression in *Pichia pastoris*

Using *Pichia* fermentation for production of recombinant proteins and enzymes, a very common strategy is to grow the culture on glycerol to a target density, and then to induce the recombinant protein synthesis by a switch to methanol. During the productive phase, methanol is rapidly consumed by the yeast and needs to be added successively in appropriate amounts to hold the concentration within a narrow range, to effectively boost the protein expression, but not to reach a toxic limit, which is very close. Therefore, a fast and reliable method for the frequent control of methanol in the culture is essential for an optimal outcome of the process.

2. Food processing

If yeast fermentation is used in food processing (e.g. for production of ethanol), the growth of methanol-producing species should be prevented. In the processing and storage of protein-rich food material (e.g. meat or watery cheese), contamination with yeasts generally has to be avoided. Sensitive testing for methanol can be an appropriate check to confirm the absence of such yeast contaminations during the processing. The high sensitivity of the Cedex Methanol assay enables early detection of yeast metabolism.

Wide concentration range for various applications

In order to provide highly accurate results over a wide methanol concentration range of 0.04 to 50 g/L, there are three instrument protocols. A low concentration range providing high sensitivity, a medium and a high range.

Protocol	Methanol concentration range
MEOHL	0.04 – 0.75 g/L, 1.25 – 23.40 mmol/L
MEOHB	0.25 – 5.00 g/L, 7.8 – 156.0 mmol/L
MEOHD	2.50 – 50.00 g/L, 78.0 – 1,560.0 mmol/L

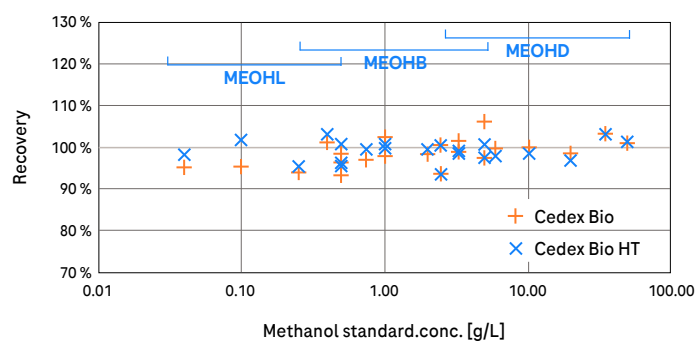
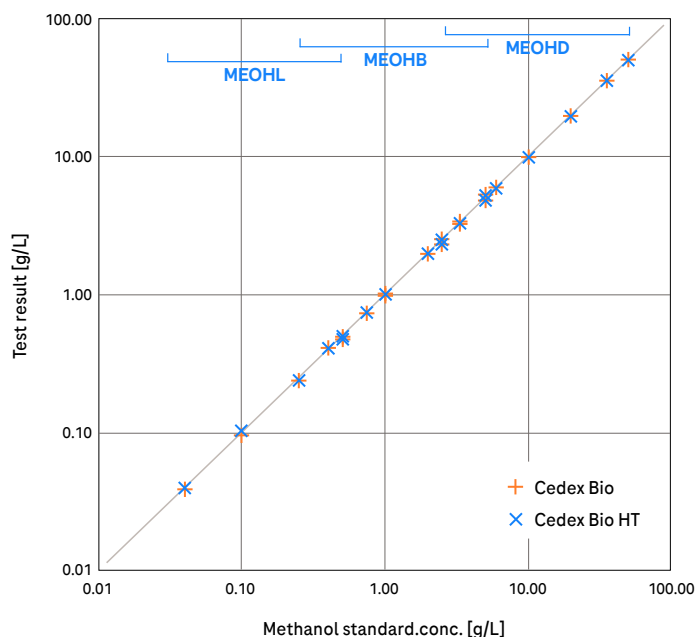
Robust and convenient assay format

The kit reagents for methanol testing are liquid-stable and ready to use, and can stand onboard for several weeks. Due to their high stability, the reagents only need to be re-calibrated after 2 months. The methanol assay uses only a low sample volume of several microliters (depending on the protocol for a low or high concentration range), saving sample material and minimizing potential interferences of other substances in the sample matrix.

	Methanol Bio	Methanol Bio HT
On-board stability	28 days	56 days
Calibration interval	56 days	56 days
Sample volume	3 – 20 µL	3 – 20 µL

Accuracy

For evaluation of the accuracy of the Cedex methanol testing, common culture media for *Pichia* fermentation were spiked with methanol concentrations spanning the whole test range and analyzed on Cedex Bio and Cedex Bio HT Analyzers.



01 + 02

Accuracy: Culture media samples spiked with methanol concentrations from 0.04 up to 50 g/L were determined on a Cedex Bio and on a Cedex Bio HT Analyzer. Over the whole range, the three protocols show a perfect linearity, and for all samples the recovery is within $\pm 10\%$ of the target value, with the majority of values even within $\pm 5\%$. The results from the two analyzers are perfectly consistent. (Verification data of Roche Diagnostics)

High precision

	Level 1	Level 2	Level 3
Conc.	0.5 g/L	2.0 g/L	3.3 g/L
CV in-run	2.0 %	1.4 %	1.0 %
CV inter-run	3.2 %	1.8 %	1.8 %

(Verification data of Roche Diagnostics)

In methanol testing, the high volatility of the analyte is a special challenge, requiring fast and reproducible processing of the samples in the analysis, to achieve a good precision. For evaluation of the precision, culture media samples were spiked with three methanol concentration levels and analyzed on a Cedex Bio HT Analyzer, 21 replicates at once in one run, and further replicates repeatedly on 10 days. The precision was always found within 3.2%.

Specificity, interferences

The assay is highly specific for methanol and does not produce false-positive signals with other alcohols or similar substances. Besides methanol, only formaldehyde generates a signal in the assay (refer to the reaction scheme), however, formaldehyde is highly reactive and will not be present in fermentation samples.

The following substances were tested for a potential interference in the assay. Very high concentrations of some substances may suppress the signal in the assay, potentially resulting in an underestimation of the methanol concentration.

No interferences were observed with:

- ≤ 20 g/L ethanol (see details for ethanol below)
- ≤ 20 g/L 2-propanol, isopropyl alcohol
- ≤ 20 g/L glycerol
- ≤ 20 g/L polyethylene glycol, PEG
- ≤ 1 g/L ethylenediaminetetraacetic acid, EDTA
- ≤ 1 g/L dithiothreitol, DTT
- ≤ 0.9 g/L methylisothiazolinone, MIT
- ≤ 0.9 g/L sodium azide
- ≤ 4 mol/L ammonium acetate
- ≤ 170 mmol/L citric acid
- ≤ 100 mmol/L tris(hydroxymethyl)aminomethane, Tris
- antifoam in a common working concentration

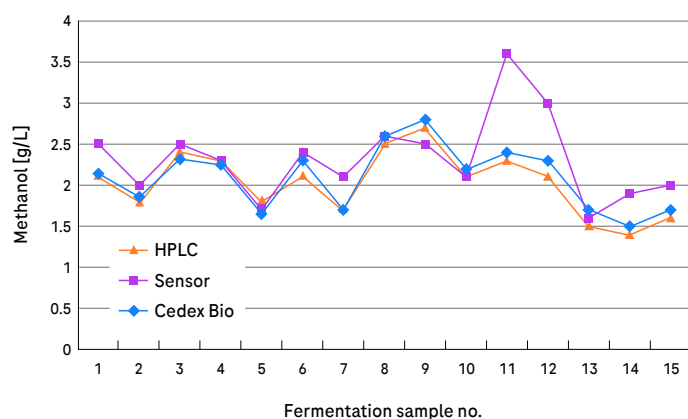
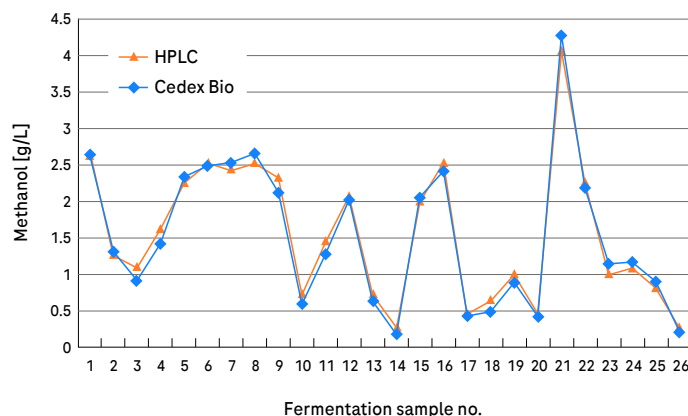
Potential interference of ethanol

In case of an ethanol concentration more than 50 times higher than the methanol concentration, there may be an underestimation of methanol, because the high ethanol decelerates the alcohol oxidase reaction in the assay. Ethanol testing is also available on the Cedex Analyzers. Accurate methanol results down to the lower limit of the measuring range can be expected with the following ethanol background concentrations.

Protocol	Methanol range	Tolerable ethanol conc.
MEOHL	0.04 – 0.75 g/L	2.0 g/L
MEOHB	0.25 – 5.00 g/L	12.5 g/L
MEOHD	2.50 – 50.00 g/L	125.0 g/L

Comparability to other methods

Established methods for determination of methanol in bioprocess control applications are HPLC and enzyme membrane sensors. Results of the Cedex Methanol test are consistent to the other methods, enabling easy transition to Cedex.



03 + 04

Method comparison: Samples from a *Pichia* fermentation process with successive methanol feed were tested for methanol in parallel using an established HPLC system and a Cedex Bio Analyzer (Fig. 03), and additionally with an enzyme sensor-based instrument (Fig. 04). The recovery of the methanol concentrations was consistent for the three methods, especially for HPLC and Cedex Bio, while the membrane sensor showed a higher imprecision. (Verification data of Roche Diagnostics Operations)

Excellent process control using Cedex® Analyzers

- The broad Cedex test menu offers assays for > 30 different analytes according to the analytical needs in various applications of cell culture, microbial fermentation and down stream processing, and the menu is permanently extended with additional assays in development.
- The Cedex Analyzers provide appropriate solutions for development labs requiring high flexibility and high throughput, and as well for qualified manufacturing environments requiring high process robustness and workflow integration.
- Cedex Bio and Cedex Bio HT Analyzers are designed for low or high sample throughput, using the same technology and the same assays with a consistent analytical performance.

Ordering information

Product	Pack size	Catalog Number
Methanol Bio ¹	4 x 50 tests	08 055 688 001
Methanol Bio HT ¹	200 tests	08 055 718 001
Calibrator M Bio ²	6 x 0.5 ml	08 083 614 001
Control M Level 1 Bio ²	6 x 0.5 ml	08 083 657 001
Control M Level 2 Bio ²	6 x 0.5 ml	08 083 665 001
Control M Level 3 Bio ²	6 x 0.5 ml	08 083 681 001

Regulatory Disclaimer

¹ For quality control/manufacturing of IVD/medical devices/pharmaceutical products only.

² For use in quality control/manufacturing process only.

Trademark

CEDEX is a trademark of Roche.



Scan for ordering
information for all Cedex
Bio Analyzer and Cedex
Bio HT Analyzer assays

© 2024

All rights reserved.

Published by

Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

[custombiotech.roche.com](https://www.custombiotech.roche.com)

Please contact your local CustomBiotech representative

Europe, Middle East, Africa, Latin America
mannheim.custombiotech@roche.com

United States
custombiotech.ussales@roche.com

Canada
custombiotech.can@roche.com

Asia Pacific
apac.custombiotech@roche.com