CUSTOMBIOTECH

Roche

Urea Assay for Cedex® Bio & Bio HT Analyzers

Reliable and convenient determination in process control

Determination of urea concentrations is needed for process control in various biomanufacturing procedures, and typical requirements are fast and accurate testing with a high automation level to minimize hands-on intervention and to enable controlled data management. Applications are for example:

- Cell cultures: Monitor health and productivity of your cell culture by accurately determining urea levels, ensuring optimal growth conditions for production of high quality biopharmaceuticals and other cell-derived products.
- Microbial fermentation: Enhance the efficiency of your fermentation process by feeding the right amount of urea for optimal nitrogen supply achieving higher product yield.
- Algae cultures: Optimize growth and productivity of algae cultures by maintaining precise control over urea levels, which is key for biofuel production, bioremediation, and other biotechnological products.
- Plant breeding: Ensure the optimal nutrient composition in liquid fertilizer for nitrogen supply in plant breeding, promoting healthy growth and development.
- Downstream processing: Streamline your procedures for purification and refolding of recombinant proteins by controlling urea levels when solubilizing protein aggregates or during protein refolding stages with decreasing urea gradients, ensuring high yield of high-quality products.

Ideal for process control

- Fully automated assay
- Ready-to-use reagents
- No sample pretreatment required
- Ability to analyze more parameters on the same platform

Wide concentration range for various applications

In order to provide highly accurate results over a wide urea concentration range of 6 to 600,000 mg/L, there are three instrument protocols. A low concentration range providing high sensitivity, a medium and a high range.

Protocol	Urea concentration range
URL	6 - 240 mg/L, 0.1 - 4 mmol/L
URB	60 - 2,400 mg/L, 1 - 40 mmol/L
URD	1,502 - 60,000 mg/L, 25 - 1,000 mmol/L, up to 600 g/L, 10 mol/L with automated dilution

For use in quality control only.

Assay principle

Urea is hydrolyzed by urease to ammonium and carbonate. The ammonium reacts with 2-oxoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH), producing L-glutamate and NAD^+. The consumption of NADH is determined by the decrease of its photometric absorption at 378 nm, which is inversely proportional to the urea concentration of the sample.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

Correction of ammonia interference

If samples contain significant amounts of free ammonia (NH_3) or ammonium ions (NH_4^+), then according to the reaction scheme above, the resulting value of the urea test in mmol/L is the sum of the actual urea concentration and half of the ammonia concentration. Therefore, if an ammonia background is expected in a sample on a relevant level compared to urea, we recommend to run an NH3 test in addition to the urea test for calculation of the accurate urea concentration:

Urea corrected = urea − 0.5 × NH3 (all values in mmol/L)

This correction can be done automatically using a ratio setting in the Cedex software. See instructions for use of the urea kit.

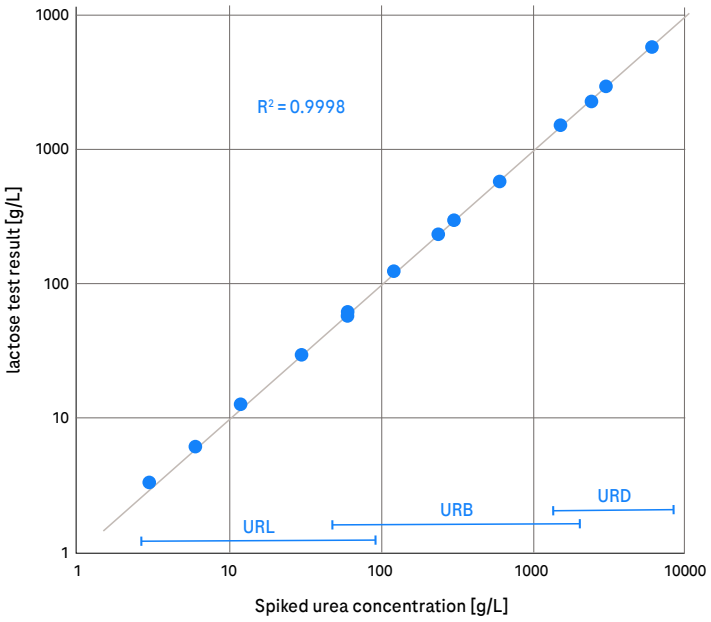
Accurate and precise measurement

The Cedex assay accurately determines urea concentrations across a wide range, as demonstrated by the perfect linearity and recovery in spiked cell culture media (see figure 01).

Representative precision data were obtained on a Cedex Bio Analyzer with samples of three urea concentration levels. Coefficients of variation (CV) were determined for in-run precision (n=21) and inter-run precision (on 10 days):

	Level 1	Level 2	Level 3
Mean [mg/L]	165	841	1,682
CV in-run	0.95 %	0.37 %	0.22 %
CV inter-run	1.81 %	1.32 %	2.15 %

(Verification data of Roche Diagnostics)



01

Accuracy. DMEM cell culture medium was spiked with a row of urea concentrations and determined on a Cedex Bio Analyzer. The three protocols URL, URB, URD were used to cover the whole concentration range. The results show a perfect linearity and a recovery within ± 5 % of the target values. (Verification data of Roche Diagnostics)

Perfect process control using Cedex Analyzers

- The Cedex Analyzers provide appropriate solutions for development labs requiring high flexibility and high through-put, and as well for qualified manufacturing environments requiring high process robustness and workflow integration.
- 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.
- Cedex Bio and Cedex Bio HT Analyzers are designed for low and high sample throughput, using the same technology and the same assays with a consistent analytical performance.

Ordering information
For determination of urea the following products are required in addition to the Cedex® Analyzer with the general system reagents and accessories:

Product	Pack size	Catalog Number
Urea Bio	4 x 50 tests	10 258 829 001
Urea Bio HT	300 tests	10 258 845 001
Calibrator A Bio	6 x 1 mL	06 682 189 001
Control A Level 1 Bio	6 x 1 mL	06 682 197 001
Control A Level 2 Bio	6 x 1 mL	06 682 227 001
Control A Level 3 Bio	6 x 1 mL	06 682 545 001

Optional for correction of ammonia background

Product	Pack size	Catalog Number
NH3 Bio	2 x 100 tests	06 343 775 001
NH3 Bio HT	150 tests	06 608 515 001
Calibrator B Bio	6 x 0.5 mL	06 682 553 001
Control B Level 1 Bio	6 x 0.5 mL	06 682 561 001
Control B Level 2 Bio	6 x 0.5 mL	06 682 570 001
Control B Level 3 Bio	6 x 0.5 mL	06 682 588 001

Regulatory disclaimers, warranty limitations and legal notes
are listed on the respective product page on custombiotech.roche.com

Trademark
CEDEX is a trademark of Roche.

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

Published by
Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

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