

Ala-Gln (GlutaMAX) Assay for Cedex Bio and Bio HT Analyzers

Reliable and convenient determination

The dipeptide Alanyl-Glutamine (Ala-Gln), also known as “GlutaMAX”, is used in cell culture media as an alternative to glutamine, which is an essential nutrient required for production of biomass and as source of energy.

Use of glutamine in cell cultures has the disadvantage of fast degradation, producing cell-toxic ammonia with negative effects on cell viability as well as protein production and glycosylation patterns.

The dipeptide Ala-Gln is heat-stable and shows no spontaneous breakdown. In a cell culture, the living cells gradually release glutamine from the dipeptide by secretion of peptidases. Depending on the number of cells and on the intensity of their metabolism, the required amount of glutamine is continuously released from the dipeptide.

For optimal culture conditions, there should always be an excess of Ala-Gln to ensure sufficient supply of glutamine, however, the excess should not be too high to avoid useless production of ammonia.

Furthermore, Ala-Gln is a relevant expense factor, and relevant cost savings can be achieved if the excess can be kept low. Therefore, a reliable and convenient method for monitoring of Ala-Gln in the culture is needed to keep the concentration in the optimal range.

Take control of your culture process

A productive cell culture period of 14 days is commonly started with a high Ala-Gln concentration, and after 7 days additional Ala-Gln is added, independent of the actual consumption, which is not known.

Now, using Cedex Analyzers for process control, the Ala-Gln concentration in the culture can be monitored and held on an optimal level by appropriate feeding, avoiding the risk of useless production of ammonia and saving costs for Ala-Gln.

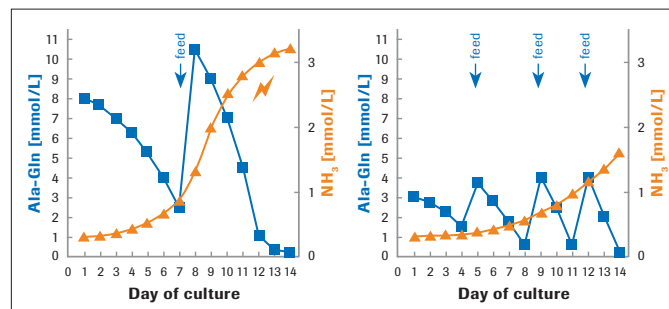
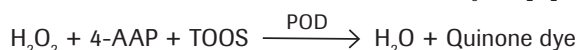
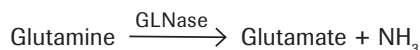
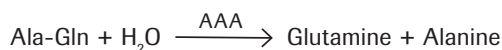


Figure 1: Use of Ala-Gln as cell culture supplement by adding standard amounts independent of the actual consumption (left), or according to the uptake by the cells monitored with a Cedex Analyzer (right). [Schematic presentation]

Assay principle

The dipeptide Ala-Gln is hydrolyzed by amino acid arylamidase (AAA) to glutamine and alanine. Glutamine is deaminated by glutaminase (GLNase) to glutamate, and oxidized by glutamate oxidase (GOD) to α -ketoglutarate, ammonia, and hydrogen peroxide, generating a colored quinone dye in presence of peroxidase (POD). The dye formation measured at 552 nm is directly proportional to the concentration of Ala-Gln.



Assay specificity

The test is not influenced by glutamate in the sample, due to a glutamate degradation step prior to addition of GLNase.

The result value is the sum of Ala-Gln and free glutamine in the sample. If the concentration of Ala-Gln only is required, then free glutamine has to be determined additionally using the glutamine test, and subtracted from the total result of the Ala-Gln test.

The AAA enzyme in this kit releases glutamine only from the dipeptide Ala-Gln. Glutamine bound in other peptides like glycyL-glutamine (Gly-Gln) or in proteins is not detected.

Benefit from the expedient assay features

	Ala-Gln Bio	Ala-Gln Bio HT
On-board stability	1 month	3 months
Calibration interval	3 months	
Measuring range	0.1 – 10 mmol/L, 21 – 2,172 mg/L up to maximal solubility with automated on-board dilution	

custombiotech.roche.com/cedex

Please contact your local CustomBiotech representative

Europe, Middle East, Africa, Latin America

Phone +49 621 759 8580
Fax +49 621 759 6385
mannheim.custombiotech@roche.com

United States

Phone +1 800 428 5433, ext. 14649 (toll-free)
Fax +1 317 521 4065
custombiotech.ussales@roche.com

Canada

Phone +1 450 686 7050
Fax +1 450 686 7012
custombiotech.can@roche.com

Japan

Phone +81 3 6634 1046
Fax +81 3 5479 0585
japan.custombiotech@roche.com

Asia Pacific

Phone +65 6371 6638
Fax +65 6371 6601
apac.custombiotech@roche.com

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Roche Diagnostics GmbH
Sandhofer Straße 116
68305 Mannheim
Germany

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Rely on precise results

	Level 1	Level 2	Level 3
Ala-Gln conc.	0.5 mmol/L	4 mmol/L	7 mmol/L
CV in-run	0.7%	0.4%	0.5%
CV inter-run	1.7%	1.4%	1.5%

Representative performance data from Cedex Bio HT Analyzers are shown. Results obtained in individual laboratories may differ. Coefficients of variation (CV) were calculated for in-run precision (n=21) and inter-run precision (on 10 days).

Ordering information

Product	Pack size	Catalog number
Ala-Gln Bio ⁽¹⁾	4 x 50 tests	08 056 978 001
Ala-Gln Bio HT ⁽¹⁾	200 tests	08 056 943 001
Calibrator D Bio ⁽²⁾	6 x 1 ml	07 368 321 001
Control D Level 1 Bio ⁽²⁾	6 x 1 ml	07 368 178 001
Control D Level 2 Bio ⁽²⁾	6 x 1 ml	07 368 186 001
Control D Level 3 Bio ⁽²⁾	6 x 1 ml	07 368 194 001

Regulatory disclaimer

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

⁽²⁾ For use in quality control/manufacturing process only.

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