



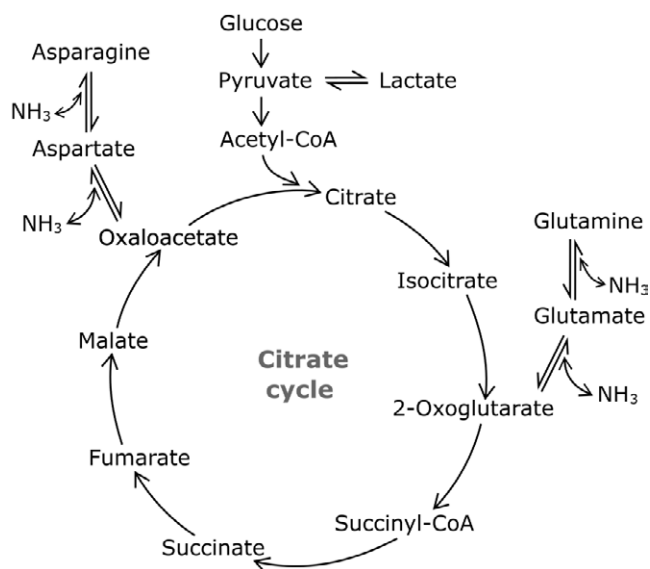
Asparagine/Aspartate Assay for Cedex Bio & Cedex Bio HT Analyzers

New tools for improved cell culture process control

Asparagine (Asn) is an important substrate in cell cultures as source of nitrogen and for the biosynthesis of new proteins, analogous to glutamine.^{1,2} It is not classified as an essential amino acid,³ nevertheless, in a culture the Asn concentration commonly decreases rapidly, and without appropriate feed cell growth and productivity may decline.^{4,5,6} Furthermore, Asn depletion causes sequence variations in recombinant proteins due to misincorporation of wrong amino acids.^{7,8} Therefore, the continuous control of Asn in a cell culture helps to achieve high yield and correct product quality.

Aspartate (Asp) also serves as substrate in a cell culture, however, similar to glutamate, Asp often accumulates as a metabolite to high concentration levels, especially if high amounts of Asn were available. Continuous monitoring of Asn and Asp enables the adequate feeding to achieve optimal culture results.

While glutamine (Gln) and glutamate (Glu) are well-established process control parameters for cell cultures, now the awareness of the importance of control of Asn and Asp progressively advances in the technical literature.



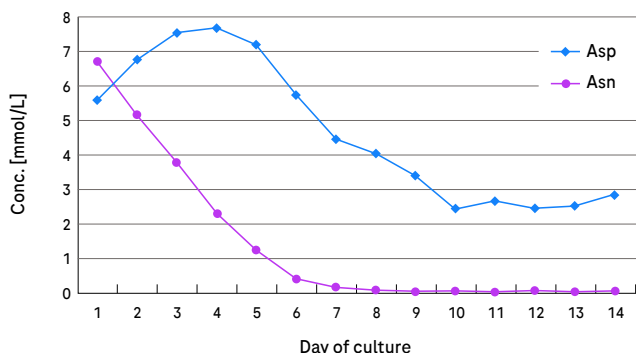
01 Excerpt from metabolic pathways² around the Citrate Cycle representing analogous roles of Asn/Asp and Gln/Glu.

Process control based on fast and reliable analytics

- Automated Cedex assays for Asn and Asp provide precision and convenience
- No sample filtration or other pretreatment required
- Wide measuring range, option for on-board dilution
- Low sample volume of 2 – 40 µL used

Application

In a cell culture, the initial amount of Asn from the fresh culture medium is rapidly consumed and the concentration decreases to a low level, limiting the productivity of the cells. Too high levels of Asn would also be suboptimal, because the cells would waste Asn and accumulate Asp. Therefore, monitoring of the Asn and Asp concentrations during the culture enables a feeding strategy maintaining the appropriate supply with Asn and Asp for optimal culture conditions.

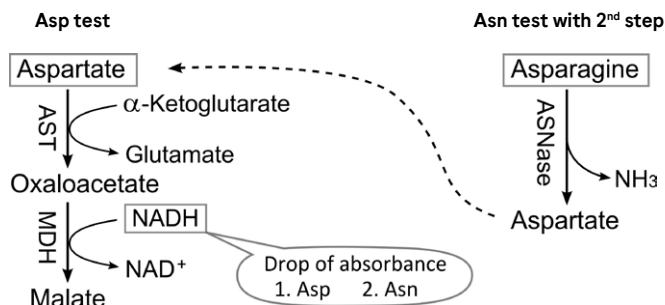


02

Asn and Asp in cell culture: The concentrations of Asn and Asp were monitored using a Cedex Bio Analyzer in an exemplary culture of CHO cells with a high starting concentration of Asn in the medium. (Roche evaluation data)

Assay principle

There is one reagent kit with all the required components, and there are several protocols selectable for the determination of Asn or Asp in the appropriate concentration ranges.



Wide test ranges

Protocol	Concentration range
Asparagine	
ASNLB (low range)	0.1 – 1 mmol/L, 13 – 132 mg/L
ASNHB (standard)	0.5 – 9 mmol/L, 66 – 1189 mg/L
ASNHD (high range)	2.5 – 45 mmol/L, 330 – 5945 mg/L, up to 225 mmol/L, 30 g/L with dilution

Protocol

Aspartate

ASPB (standard)

Concentration range

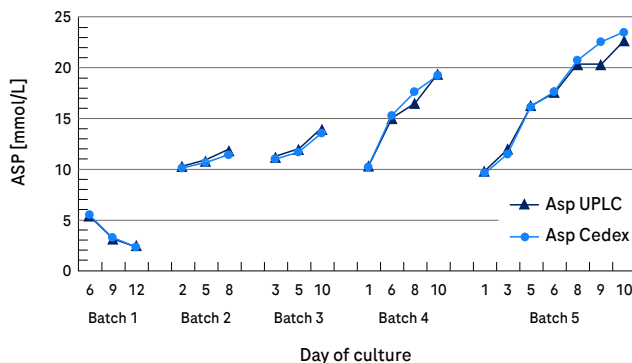
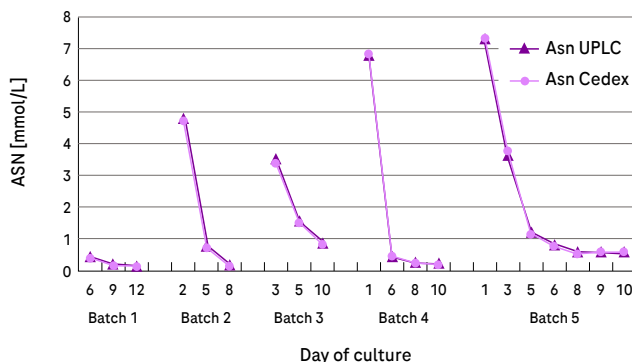
0.1 – 9 mmol/L, 13 – 1189 mg/L

ASPD (high range)

1 – 45 mmol/L, 132 – 5945 mg/L, up to 225 mmol/L, 30 g/L with dilution

Comparable to HPLC analysis

So far, the most common method for determination of Asn and Asp in culture media is HPLC or UPLC (High or Ultra Performance Liquid Chromatography). In our evaluation, a side-by-side analysis of cell culture samples using an established UPLC system and a Cedex Bio HT Analyzer for determination of the two amino acids showed highly consistent results of the two methods.

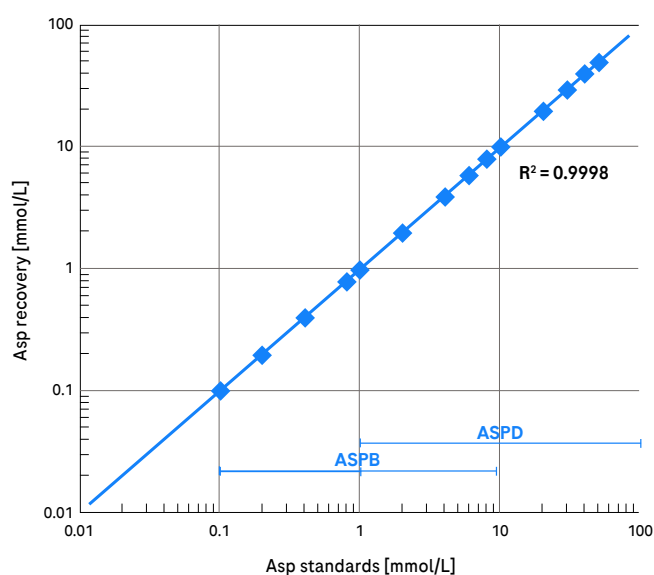
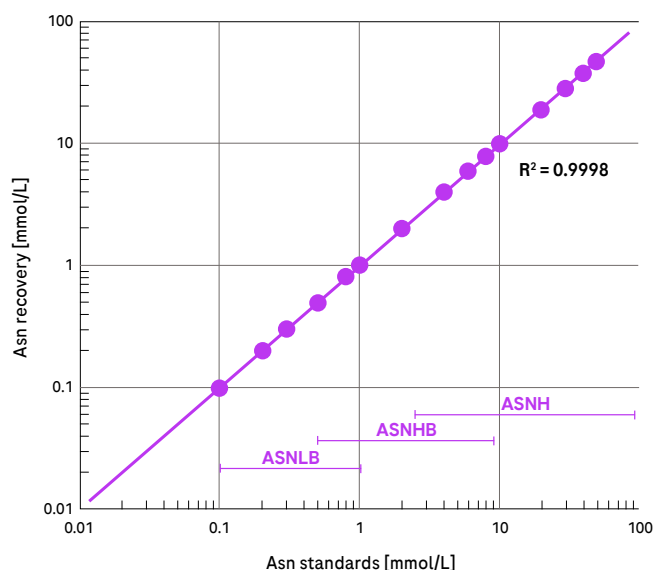


03 + 04

Asparagine/ Aspartate: Samples of the conditioned media were taken on several days from five CHO cell cultures using different media formulations with relatively high concentrations of Asn and Asp. The samples were analyzed for Asn and Asp using UPLC and a Cedex Bio HT Analyzer in parallel. The results show a good consistency of the two methods. Except of two apparent outliers of the 'Asp UPLC' values, the deviation between UPLC and Cedex results was always < 5% in these experiments. (Roche evaluation data)

High accuracy

Cell culture medium was spiked with increasing amounts of Asn and Asp and the concentrations were determined on Cedex Analyzers. The recovery of the standards shows a perfect linearity of the tests over the wide concentration range and a perfect accuracy.



05 + 06

Asparagine/ Aspartate: Asn and Asp were determined on a Cedex Bio Analyzer in a row of standards with increasing Asn or Asp concentrations, respectively, spiked into DMEM cell culture medium. According to the concentrations, the adequate test protocols were used. The results show an accurate recovery of all the standards with deviations of < 5 % from the target concentrations. (Roche evaluation data)

High precision

Asn and Asp were determined in spiked culture media samples on a Cedex Bio HT Analyzer. Coefficients of variation (CV) are calculated for 21 replicates in one run, and repeated determination on 10 days. (Roche evaluation data)

	Level 1	Level 2	Level 3
Asn			
conc. [mmol/L]	5.62	10.6	16.4
CV in-run	0.6 %	2.8 %	1.9 %
CV, inter-run	3.6 %	2.8 %	3.2 %
Asp			
conc. [mmol/L]	62	10.6	16.4
CV in-run	4.6 %	2.8 %	1.9 %
CV, inter-run	3.6 %	2.8 %	3.2 %

Potential interferences

With samples containing much higher concentrations of Asp compared to Asn, the determination of Asn may be influenced by a limitation of the NADH substrate (refer to assay principle). However, the Asn test protocols have an automated check for that effect, and in case of a risk of an influence on the Asn determination, the result will be flagged as “High Act” by the software. Then the sample should be re-tested using a protocol for a higher Asn range, tolerating higher Asp background concentrations.

The following table shows the limits of the Asp concentrations which will not influence the determination of Asn:

Asn protocol	Asn range	Asp tolerance
ASNLB (low)	0.1 – 1 mmol/L	< 7 mmol/L
ASNHB (std.)	0.5 – 9 mmol/L	< 13 mmol/L
ASNHD (high)	2.5 – 45 mmol/L	< 65 mmol/L

(Roche evaluation data)

Ordering information

For determination of Asn or Asp the following products are required in addition to the Cedex Analyzer with the general system reagents and accessories:

Product	Pack size	Catalog Number
Asn/Asp Bio *	4 x 50 tests	09 437 312 001
Asn/Asp Bio HT *	200 tests	09 437 339 001
Calibrator C Bio **	6 x 1 mL	07 020 716 001
Control C Level 1 Bio **	6 x 1 mL	07 020 724 001
Control C Level 2 Bio **	6 x 1 mL	07 020 872 001
Control C Level 3 Bio **	6 x 1 mL	07 020 902 001

Regulatory Disclaimer

* For use in quality control/manufacturing process only.

** For quality control/manufacturing of IVD/medical devices/
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