N-terminal Sequencing – 5 & 10 Residues

By Edman Degradation

Order 12345

John Doe

ABCDE Pharma Inc.

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Samples received

The following samples were received at Alphalyse for protein analysis.

Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6

Objective

Determination of the N-terminal amino acid sequence by Edman degradation – 5 & 10 residues.

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Analytical Procedure

Introduction

The procedure determines the N-terminal amino acid sequence of proteins and peptides by the Edman degradation chemistry.

Sample preparation

The samples were shipped to Alphalyse pre-blotted on PVDF membranes stained with CBB. For the individual analyses all of the provided sample was used.

Experimental

N-TERMINAL SEQUENCING BY EDMAN DEGRADATION
The analysis is performed on an ABI Procise 494 sequencer. The sample can be on a PVDF membrane or in-solution and loaded on an acid-etched glass fiber disk. The Edman degradation is a cyclic procedure where amino acid residues are cleaved off one at a time and identified by chromatography. There are 3 steps in the cyclic procedure. In step 1 the PITC reagent is coupled to the N-terminal amino group under alkaline conditions. In step 2 the N-terminal residue is cleaved in acidic media. In step 3, the PITC coupled residue is transferred to a flask, converted to a PTH-residue and identified by HPLC chromatography. The next cycle is then started for identification of the next N-terminal residue.

DATA INTERPRETATION
In the N-terminal sequencing procedure by Edman degradation it should be noted that:
- If the protein is N-terminally blocked by for example an acetyl group or pyroglutamate residue, then the amino group is unavailable for the PITC coupling. No sequence will be seen.
- Cysteine and post-translationally modified amino acid residues will give a blank result for this residue.
- If the sample contains more than one protein, or N-terminal variants of the same protein, then 2 or more residues will be identified in each cycle and shown as X/Y/Z in the report.
- Due to differences in the chemical reaction for certain amino acids the identification of some residues may be uncertain and shown in parentheses (X/Y) in the report.
- The cyclic process is not always 100% and therefore carryover from previous cycle can be observed. The identity of each single residue is detected by subtracting the previous chromatogram with the present chromatogram.
- The raw chromatography data are enclosed in the Appendix. In the raw data, Cycle 1 is a blank run, Cycle 2 is the amino acid standard showing the retention time and response for all the amino acids. Cycle 3 is the first cycle of the real sample, corresponding to the N-terminal amino acid. "

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Results

Sample name: Sample 1

Sample information:	PVDF
Cycles:	5
Raw Data File Name:	P1AB1234_12345_Sample1.pdf
Signal strength:	Strong
Confidence:	High

Cycle	Residue	Amino Acid
1	Blank	-
2	Standard	-
3	1	V
4	2	E
5	3	P
6	4	K
7	5	S

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Sample name: Sample 2

Sample information:	PVDF
Cycles:	5
Raw Data File Name:	P1AB1234_12345_Sample2.pdf
Signal strength:	Good
Confidence:	High

Cycle	Residue	Amino Acid
1	Blank	-
2	Standard	-
3	1	M
4	2	H
5	3	H
6	4	H
7	5	H
8	6	H
9	7	H
10	8	G
11	9	S
12	10	G

Comments: The results show coherence with the provided sequence and there is no apparent truncation of the N-terminal.

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Sample name: Sample 3

Sample information:	PVDF
Cycles:	5
Raw Data File Name:	P1AB1234_12345_Sample3.pdf
Signal strength:	Strong
Confidence:	Good

Cycle	Residue	Amino Acid
1	Blank	-
2	Standard	-
3	1	S/V/K
4	2	Y/E
5	3	E/P
6	4	L/(K)
7	5	T/(S)

Comments: The sample showed a mixture of signals, which also was expected due to the sample containing fragments of an antibody and light chain.

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Sample name: Sample 4

Sample information:	PVDF
Cycles:	5
Raw Data File Name:	P1AB1234_12345_Sample4.pdf
Signal strength:	Strong
Confidence:	High

Cycle	Residue	Amino Acid
1	Blank	-
2	Standard	-
3	1	V
4	2	E
5	3	P
6	4	K
7	5	S

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Sample name: Sample 5

Sample information:	PVDF
Cycles:	5
Raw Data File Name:	P1AB1234_12345_Sample5.pdf
Signal strength:	Fine
Confidence:	High

Cycle	Residue	Amino Acid
1	Blank	-
2	Standard	-
3	1	V
4	2	E
5	3	P
6	4	K
7	5	S

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Sample name: Sample 6

Sample information:	PVDF
Cycles:	5
Raw Data File Name:	P1AB1234_12345_Sample6.pdf
Signal strength:	n/a
Confidence:	n/a

Cycle	Residue	Amino Acid
1	Blank	-
2	Standard	-
3	1	-
4	2	-
5	3	-
6	4	-
7	5	-

Comments: The sample appears to be N-terminally blocked, since there is no signal despite the apparent high amount of loaded sample. Since the samples are antibody fragments, it is a possibility that the N-terminal residue is pyroglutamic acid (pyro-Q), which blocks the Edman chemistry.

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