
Test report	224319	10.01.2023
Customer	DERMO Gıda Karacaoglan Mahallesi 6167 Sokak No. 40 / A-A Isikkent / Bornova / Izmir Türkiye / Türkei	
Order	Protein Identification via LC-MS	
Date of order / delivery	25.11.2022	
Test sample / amount	Egg shell membrane	
Sampling	by customer, test pieces from material by FILK gGmbH	
Test methods	see test results, climate for conditioning and physical testing: 23 ± 2 °C, 50 ± 5 % relative humidity	

Protein Identification via LC-MS according FILK-AA-751.44 (2022-05)

For LC-MS analysis, the sample was enzymatically digested with trypsin. The resulting peptides were purified and separated on a reversed phase column using high performance liquid chromatography. The eluting peptides were analyzed on-line by tandem mass spectrometry using an ion trap instrument (QTRAP 4000, Sciex). Spectra were recorded by the Analyst program (Sciex) recording two product ion scans per full MS scan. The resulting peak lists were analysed as follows:

The software ProteinPilot (ABSciex) with the paragon algorithm was used applying the following parameters:

Software Version:	ProteinPilot™ Software 4.5
Revision Number:	1656
Paragon™ Algorithm:	4.5.0.0, 1654
Sample Type:	Identification
Cys. Alkylation:	None
Digestion:	Trypsin
Instrument:	4000 QTRAP ESI
Special Factors:	-
ID Focus:	Biological modifications
Database:	uniprot-rattus+rattus+cont
Search Effort:	Thorough
FDR Analysis:	Yes
Detected Protein Threshold [Unused ProtScore (Conf)] >:	3.00 (99.9%)
Competitor Error Margin (ProtScore):	2.00

The database *uniprot-gallus+gallus+domesticus+cont.fasta* contains all entries found in the uniprot database (23.09.2021) with the search term *Gallus gallus*. The database includes a contaminant database provided by Sciex (Swissprot and NCBI entries). Only proteins for which at least two peptides could be matched are regarded as identified.

Results:

For testing of the egg shell membrane, a double determination was carried out. Four proteins were identified:

- (1) the α_1 chain of type X collagen
- (2) a cellular glycoprotein containing the D-domain of the von Willebrand factor, which is active in mammalian cells in the context of blood clotting
- (3) the enzyme lysyl oxidase, which plays a role in the cross-linking of extracellular matrix proteins
- (4) the enzyme lysozyme, which participates in the hydrolysis of peptidoglycans

The proteins (1) and (2) were those two with the highest number of peptides identified via LC-MS/MS measurement.

Peptides, which can be assigned to other proteins, were not detected.

In addition, bovine trypsin was found in the sample material. This enzyme was used during digestion of the egg shell membrane as part of sample preparation and, therefore, is to be regarded as a contamination.

Collagen type V concentration is too low in the tested egg shell membrane.

The results are summarised in table 1.

Explanations of the table captions:

N:	Rank of protein
Score Unused:	Sum of the single peptide confidence scores (only spectra which have not been used for a higher ranking protein)
Score Total:	Total protein score
% Cov (95):	Part of the protein sequence that was covered by peptides having a confidence of higher 95%
Accession:	Accession number of NCBI (gi)
Peptides (95%):	Number of identified peptides with a confidence above 95%

Table 1: Results for LC-MS/MS analysis using the database uniprot-gallus+gallus+domesticus+cont.fasta

Egg shell membrane_1							
N	Unused	Total	% Cov (95)	Accession #	Name	Species	Peptides (95%)
1	8.04	8.04	7.6	tr A0A3Q2UC95 A0A3Q2UC95_CHICK	VWFD domain-containing protein OS=Gallus gallus OX=9031 PE=4 SV=1	CHICK	9
Egg shell membrane_2							
N	Unused	Total	% Cov (95)	Accession #	Name	Species	Peptides (95%)
1	31.51	31.51	12.7	tr A0A3Q2UC95 A0A3Q2UC95_CHICK	VWFD domain-containing protein OS=Gallus gallus OX=9031 PE=4 SV=1	CHICK	20
2	25.3	25.3	23.2	sp P08125 COAA1_CHICK	Collagen alpha-1(X) chain OS=Gallus gallus OX=9031 GN=COL10A1 PE=1 SV=4	CHICK	14
3	8.23	8.23	5	tr A0A0A0MQ32 A0A0A0MQ32_CHICK	Lysyl oxidase homolog OS=Gallus gallus OX=9031 GN=LOXL2 PE=3 SV=1	CHICK	4
4	5.1	5.1	19.1	tr B8YK77 B8YK77_GALLA	Lysozyme C OS=Gallus lafayetteii OX=9032 GN=LYZ PE=3 SV=1	GALLA	4
5	3.63	3.63	15.6	cont 000137	spt P00760 Cationic trypsin precursor (EC 3.4.21.4) (Beta- trypsin) (Fragment) [Bos taurus (contaminant)]	Bos taurus (contaminant)	3

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