Art no: 05-0104

Lot no: 280809

CERTIFICATE OF ANALYSIS

Product name:	***Lens culinaris* lectin, *Lens culinaris* agglutinin**
Production date:	2022-02-24
Date of release:	2022-04-21
Stability:	2027-02
Form:	Lyophilized

Analysis	Specification	Result
Appearence	White powder or flocculate by visual inspection resulting in a clear solution.	Fulfills requirement
Solubility	Dissolves in distilled water and 0.9% NaCl within 5 minutes.	Fulfills requirement
Assay (%)	≥ 85 % protein by OD_{280} nm (ε 1mg/ml = 1,34), essentially salt free.	87,8 %, fulfills requirement
Electrophoresis	Gives two major bands in isoelectroforesis corresponding to the two isomers LCA-A and LCA-B.	Fulfills requirement, see Bilaga 1.
Activity Haemagglutination/inhibition	Agglutinates human erythrocytes (2% blood solution) when lectin concentration is ≤ 10 µg/ml in 0.9 % NaCl after 1 hour at 25 °C. inhibition appears with 60 mM Methyl mannoside (end conc. 20 mM) at a titer minimum 4 steps lower than titer of control. Control must have a titer of minimum 32.	Titer inhibition with 20 mM Methyl mannoside = 2; Agglutination at 2,6 µg/ml. Fulfills requirement, see Bilaga 3.
UV scan	Typical appearance of Lens culinaris lectin at 700-230 nm.	Fulfills requirement, see Bilaga 2.
Bacterial burden	Less than 10^2 CFU per 1 g of solid substance.	< 100 CFU/g, fulfills requirement

Bilaga:
1. IEF electrophoresis.
2. UV Scan 700-230nm.
3. Activity, Haemagglutination control.

The above material has met all quality specifications and has been reviewed by a quality representative.

Quality Assurance, Max Johansson

2022-04-21

Date

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Medicago

Bilaga 1, tillhör M-00018

IEF analysis *Lens culinaris* lectin, Lot 280809

Isoelectric focusing using Pharmacia Phastsystem (Amersham Biosciences).

Materials

Phastgel IEF 3-9

Reference proteins pH 3.5 – 9.3 (IEF Stdrd)

Amylglucosidase -	3.50 pI
Methyl red (dye) -	3.75 pI
Trypsin inhibitor -	4.55 pI
b-Lactoglobulin A -	5.20 pI
Carbonic anhydrase B (bovine) -	5.85 pI
Carbonic anhydrase B (human) -	6.55 pI
Myoglobin, acidic band -	6.85 pI
Myoglobin, basic band -	7.35 pI
Lentil lectin, acidic -	8.15 pI
Lentil lectin, middle -	8.45 pI
Lentil lectin, basic -	8.65 pI
Trypsinogen -	9.30 pI

Method

The proteins were diluted to 4 mg/ml in milliQ water supplemented with 30 % saccarose. The gels were fixed for 10 min, washed and stained with IEF staining sol. for 10 min at 50°C and then destained.

Program

Sample applicator down at			2.2	0Vh		
Sample applicator up at			2.3	0Vh		
SEP	**2.1**	2000V	2.5mA	3.5W	15°C	75Vh
SEP	**2.2**	200V	2.5mA	3.5W	15°C	15Vh
SEP	**2.3**	2000V	2.5mA	3.5W	15°C	410Vh

Result

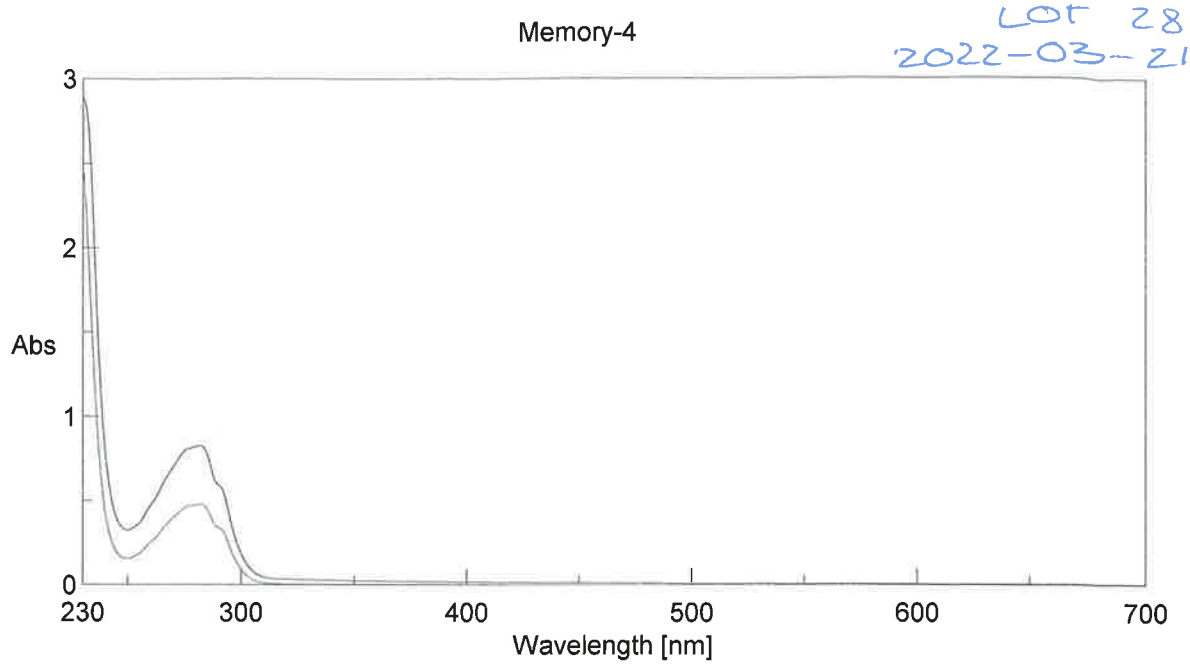
1 2 3 4 5 6 7 8

Lane 1,8	**Empty**
Lane 2,7	IEF standard pH 3.5 – 9.3
Lane 3-4	LCA lot 280809
Lane 5-6	LCA Ref lot 273215

Analysis performed by Cynthia Hassinen

Cynthia Hassinen, 2022-03-29

Bilaga 2
Tillhör M-0009
LOT 280809
2022-03-21/CH



Memory-2
Memory-4

[Comments]	
Sample name	LCA, referens
Comment	lot 273215
User	CH
Division	QC
Company	Medicago
[Detailed Information]	
Creation date	2022-03-21 12:24
Date modified	2022-03-21 12:26
Data array type	Linear data array
Horizontal axis	Wavelength [nm]
Vertical axis	Abs
Start	700 nm
End	230 nm
Data interval	0.5 nm
Data points	941
[Measurement Information]	
Instrument name	Jasco UV Vis
Model name	V-760
Serial No.	A011061800
Accessory	USE-753
Accessory S/N	A011061800
Cell	
Ref. beam	
Measurement date	2022-03-21 12:24
Parameter file	C:\Program Files (x86)\JASCO\SpectraManager\samples\230-700.uvsp
Photometric mode	Abs
Measurement range	700 - 230 nm
Data interval	0.5 nm
Bandwidth	2.0 nm
Response	0.06 sec
Scan speed	1000 nm/min
Change source at	340 nm
Light source	D2/WI

Medicago

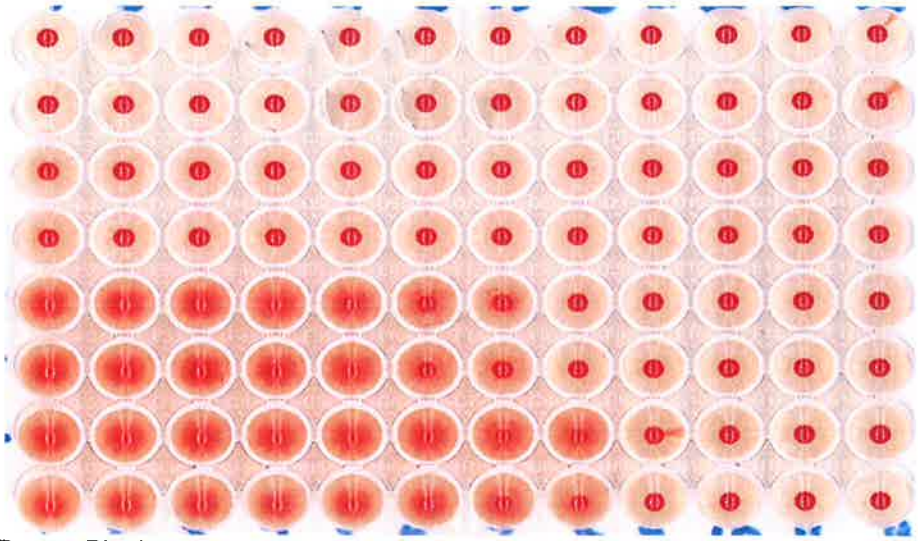
+

Bilaga 3, tillhör M-00020

Haemagglutination (human blood, group 0), Lot 280809

Lens culinaris agglutinin (LCA)

Column	1	2	3	4	5	6	7	8	9	10	11	12	
Conc	333	167	83	42	21	10	5.2	2.6	1.3	0.6	0.3	0.16	µg/mL
Titer	0	2	4	8	16	32	64	128	256	512	1024	2048	



Row A,B	Blank
Row C,D	With 20 mM α-metyl-D-mannosid
Row E,F	With 2 mM α-metyl-D-mannosid
Row G,H	Only lectin without α-metyl-D-mannosid (agglutination control)

Plate scanned:

Signature: Cynthia Hassinen

Cynthia Hassinen 2022-03-23