

Art no: 05-0104

# CERTIFICATE OF ANALYSIS

Lot no: 264427



Intertek

Product name:	<b><i>Lens culinaris</i> lectin, <i>Lens culinaris</i> agglutinin</b>
Production date:	2021-03
Date of release:	2021-04-09
Stability:	2026-03
Form:	Lyophilized

Analysis	Specification	Result
Appearance	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 <sub>280</sub> nm (ε 1mg/ml = 1,34), essentially salt free.	89,7 %, fulfills requirement
Electrophoresis	Gives two major bands in isoelectroforesis corresponding to the two isomers LCA-A and LCA-B.	Fulfills requirement, see attachment 3.
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. Total 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 = 1; Agglutination at 10 µg/ml. Fulfills requirement, see attachment 2.
UV scan	Typical appearance of <i>Lens culinaris</i> lectin at 700-230 nm.	Fulfills requirement, see attachment 1.
Bacterial burden	Less than 10 <sup>2</sup> CFU per 1 g of solid substance.	< 100 CFU/g, fulfills requirement

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

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

Quality Assurance, Monika Scuka

2021-04-09

Date

Bilaga 1  
Tillhör M-00019

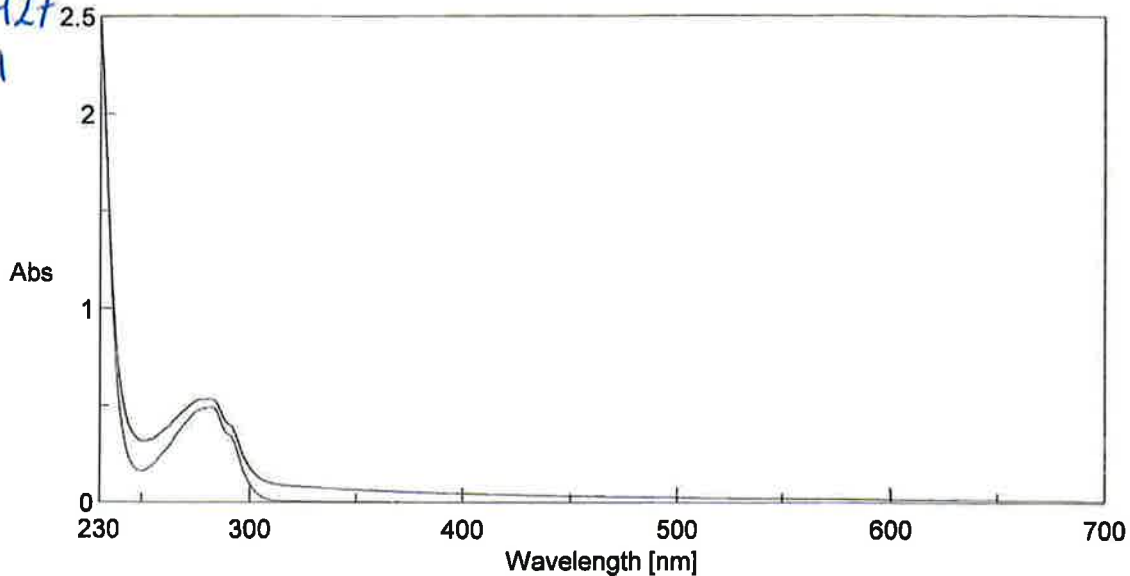
APPENDIX 1

Lotnr: 264427

2021-03-31

/MeBr

Memory-2



[Comments]

Sample name LCA lot 264427  
Comment  
User  
Division  
Company Medicago

Memory-2  
Memory-4

[Detailed Information]

Creation date 2021-03-31 13:41  
Date modified 2021-03-31 13:42

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 2021-03-31 13:41

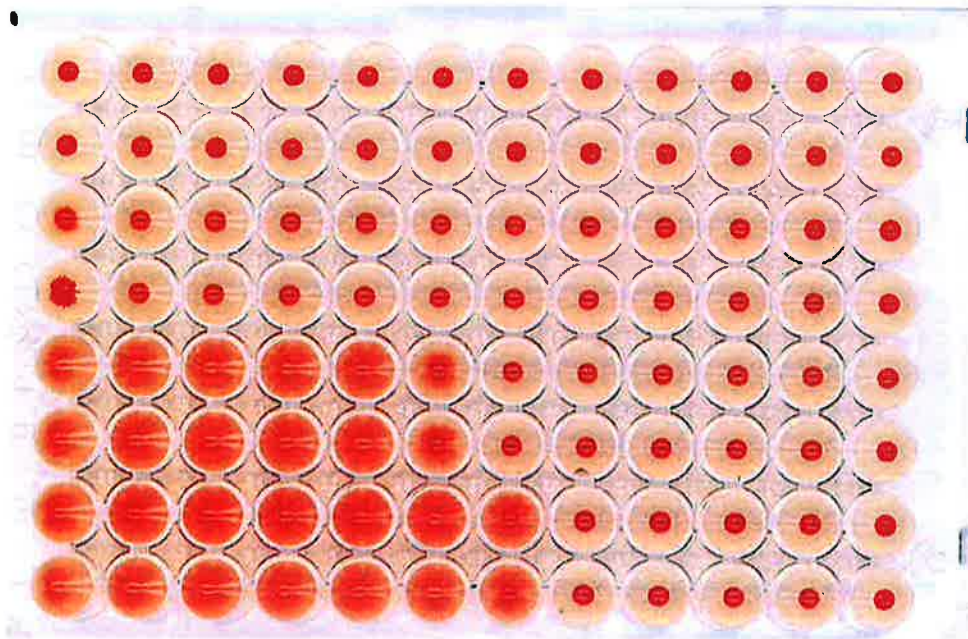
Parameter file C:\Program Files (x86)\JASCO\SpectraManager\samples\230-700.uvsp  
Photometric mode Abs  
Measurement range 700 - 230 nm  
Data interval 0.5 nm  
Bandwidth 2.0 nm  
Response 0.06 sec  
Scan speed 1000 nm/min  
Change source at 340 nm  
Light source D2/WI

**Bilaga 3, tillhör M-00020**

**Haemagglutination (human blood, group 0), Lot 264427**

***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  $\alpha$ -metyl-D-mannosid  
 Row E,F      With 2 mM  $\alpha$ -metyl-D-mannosid  
 Row G,H      Only lectin without  $\alpha$ -metyl-D-mannosid (agglutination control)

Plate scanned:

Date: 2021-04-01 / SA

Signature:   
 Salah Ahmed

Bilaga 1, tillhör M-00018

**IEF analysis *Lens culinaris* lectin, Lot 264427**

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 % saccharose. The gels were fixed for 3 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	IEF standard pH 3.5 – 9.3
Lane 2-3	Ref lot 203915
Lane 4-5	LCA lot 271101
Lane 6-7	LCA lot 264427
Lane 8	Empty

Analysis performed by



Martina Brolin , 2021-03-31