


LAMPIRAN

Lampiran 2. Au foil 24 karat



## Lampiran 3. Sertifikat analisa asam askorbat



## Certificate of Analysis

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1.00468.0000 L(+)-Ascorbic Acid for analysis EMSURE® ACS, Reag. Ph Eur  
Batch K54197668

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	Spec. Values		Batch Values	
Assay (iodometric)	99.0 - 100.5	%	99.7	%
Identity (IR-spectrum)	conforms		conforms	
Appearance	white or almost white, crystalline powder		white or almost white, crystalline powder	
Appearance of solution (50 g/l CO <sub>2</sub> -free water)	clear (≤ 3 NTU) and not so intense in colour than reference solution BY <sub>r</sub>		clear (≤ 3 NTU) and not so intense in colour than reference solution BY <sub>r</sub>	
pH (50 g/l CO <sub>2</sub> -free water)	2.1 - 2.6		2.4	
Spec. rotation [α] <sub>D</sub> (100 g/l, water)	+20.5 - +21.5	*	+20.8	*
Chloride (Cl)	≤ 50	ppm	≤ 50	ppm
Sulfate (SO <sub>4</sub> )	≤ 20	ppm	≤ 20	ppm
Cu (Copper)	≤ 5	ppm	≤ 5	ppm
Fe (Iron)	≤ 2	ppm	≤ 2	ppm
Heavy metals (ACS)	≤ 10	ppm	≤ 10	ppm
Oxalic acid	≤ 0.2	%	≤ 0.2	%
Related substances (HPLC) (Impurity C)	≤ 0.15	%	0.01	%
Related substances (HPLC) (Impurity D)	≤ 0.15	%	< 0.05	%
Related substances (HPLC) (unspecified impurities singly)	≤ 0.10	%	0.06	%
Related substances (HPLC) (sum of impurities (except impurity C and D))	≤ 0.2	%	< 0.1	%
Sulfated ash (600 °C)	≤ 0.05	%	≤ 0.05	%
Loss on Drying (105 °C)	≤ 0.1	%	< 0.1	%

Date of release (DD.MM.YYYY) 17.03.2022  
Minimum shelf life (DD.MM.YYYY) 31.03.2024

Dr. Sebastian Lips  
Responsible laboratory manager quality control


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Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt (Germany): +49 6151 72-0  
EMD Millipore Corporation - a subsidiary of Merck KGaA, Darmstadt, Germany  
400 Summit Drive, Burlington, MA 01803, USA, Phone +1 (781) 533-6000  
SALSA Version 1179899/950000922577/ Date: 17.03.2022

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Lampiran 4. Sertifikat analisa enzim tirosinase



3050 Spruce Street, Saint Louis, MO 63103, USA  
 Website: [www.sigmaaldrich.com](http://www.sigmaaldrich.com)  
 Email USA: [techserv@sial.com](mailto:techserv@sial.com)  
 Outside USA: [eurtechserv@sial.com](mailto:eurtechserv@sial.com)

### Certificate of Analysis

**Product Name:** Tyrosinase from mushroom - lyophilized powder,  $\geq 1000$  unit/mg solid


**Product Number:** T3824  
**Batch Number:** SLCG8506  
**Brand:** SIGMA  
**CAS Number:** 9002-10-2  
**MDL Number:** MFCD00082118  
**Storage Temperature:** Store at -20 °C  
**Quality Release Date:** 23 SEP 2020  
**Recommended Retest Date:** SEP 2023

Test	Specification	Result
units/mg Solid Tyrosinase Activity Unit Definition: One unit will cause an increase in A280 of 0.001 per minute at pH 6.5 at 25 deg C in a 3 mL reaction mix containing L-tyrosine	$\geq 1000$	8503
units/mg Solid Polyphenol Oxidase Activity Unit Definition: One unit will cause a decrease in A265 of 0.001 per minute at pH 6.5 at 25 deg C. In a 3 mL reaction mixture containing L-Beta-3,4-dihydroxyphenyl-alanine (L-DOPA)	$\geq 30000$	300628
units/mg Solid Catechol Oxidase Activity Unit Definition: One unit will cause a decrease in A265 of 0.001 per minute at pH 6.5 at 25 deg C in a 3 mL reaction mixture containing catechol and ascorbic acid	$\geq 30000$	1778114

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

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(Lanjutan)



3050 Spruce Street, Saint Louis, MO 63103, USA  
Website: [www.sigmaldrich.com](http://www.sigmaldrich.com)  
Email USA: [techserv@sial.com](mailto:techserv@sial.com)  
Outside USA: [eurtechserv@sial.com](mailto:eurtechserv@sial.com)

### Certificate of Analysis

Product Number: T3824  
Batch Number: SLCG8506

---


  
Brian Dulle, Supervisor  
Quality Assurance  
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.



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Lampiran 5. Sertifikasi analisa  $\text{KH}_2\text{PO}_4$ 



## Certificate of Analysis

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1.04873.0000 Potassium dihydrogen phosphate for analysis EMSURE® ISO  
 Batch AM1704373

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	Spec. Values		Batch Values	
Assay (alkalimetric, calculated on dried substance)	99.5 - 100.5	%	99.8	%
Assay (alkalimetric; dried substance)	≥ 99.5	%	99.9	%
pH-value (5 %; water)	4.2 - 4.5		4.3	
Chloride (Cl)	≤ 0.0005	%	≤ 0.0005	%
Sulfate (SO <sub>4</sub> )	≤ 0.003	%	≤ 0.003	%
Total nitrogen (N)	≤ 0.001	%	≤ 0.001	%
Heavy metals (as Pb)	≤ 0.0010	%	≤ 0.0010	%
As (Arsenic)	≤ 0.0002	%	≤ 0.0002	%
Cu (Copper)	≤ 0.0003	%	≤ 0.0003	%
Fe (Iron)	≤ 0.0010	%	≤ 0.0010	%
Na (Sodium)	≤ 0.02	%	≤ 0.02	%
Pb (Lead)	≤ 0.001	%	≤ 0.001	%
Reducing substances	passes test		passes test	
Loss on drying (110 °C)	≤ 0.2	%	< 0.2	%
Loss on drying (130 °C)	≤ 0.2	%	< 0.1	%

Corresponds to ISO

Date of release (DD.MM.YYYY) 07.05.2021  
 Minimum shelf life (DD.MM.YYYY) 31.01.2026

Claudia Wiegand  
 Responsible laboratory manager quality control

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Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt (Germany): +49 6151 72-0  
 EMD Millipore Corporation - a subsidiary of Merck KGaA, Darmstadt, Germany  
 400 Summit Drive, Burlington, MA 01803, USA, Phone +1 (781) 533-6000  
 SALSA Version 1073438/990000829993/ Date: 07.05.2021

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## Lampiran 6. Sertifikat analisa L-tirosin

**SIGMA-ALDRICH**

3050 Spruce Street, Saint Louis, MO 63103 USA  
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

**Certificate of Analysis**

**Product Name:** L-Tyrosine  
reagent grade, >= 98 % HPLC

**Product Number:** T3754

**Batch Number:** BCCC5665

**Brand:** Sigma-Aldrich

**CAS Number:** 60-18-4

**Formula:** 4-(HO)C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>CH(NH<sub>2</sub>)CO<sub>2</sub>H

**Formula Weight:** 181.19

**Quality Release Date:** 16 DEC 2019

**Recommended Retest Date:** NOV 2025

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	WHITE TO OFF WHITE	OFF-WHITE
APPEARANCE (FORM)	POWDER	POWDER
PURITY (HPLC AREA %)	≥ 98 %	100 %
SPECIFIC ROTATION	-11.2 - -9.8 DEGREES	-10.5 DEGREES
CONCENTRATION	C=5 IN 1M HYDROCHLORIC ACID AT 25 C	C=5 IN 1M HYDROCHLORIC ACID AT 25 C
SOLUBILITY (COLOR)	COLORLESS TO LIGHT YELLOW	COLORLESS
SOLUBILITY (TURBIDITY)	CLEAR	CLEAR
SOLUBILITY (METHOD)	50MG/ML IN 1M HYDROCHLORIC ACID	50MG/ML IN 1M HYDROCHLORIC ACID
CARBON CONTENT	58.5 - 60.8 %	59.5 %
NITROGEN CONTENT	7.4 - 8.0 %	7.7 %
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

*Dr. R. Schwenninger*

Dr. Reinhold Schwenninger  
Quality Assurance  
Buchs, Switzerland

Sigma-Aldrich warrants that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Sigma-Aldrich Certificate of Analysis - Product T3754 Lot BCCC5665 Page 1 of 1

Lampiran 7. Sertifikat analisa asam kojat



**Certificate Of Analysis**

Item Number	K1062	Lot Number	1J0323
Item	Kojic Acid		
CAS Number	501-30-4		
Molecular Formula	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	Molecular Weight	142.11

Test	Specification		Result
	min	max	
ASSAY	99 %		99.4 %
HEAVY METALS (as Pb)		20 ppm	<10 ppm
ARSENIC (As)		2 ppm	<0.1 ppm
LOSS ON DRYING		0.6 %	0.35 %
RESIDUE ON IGNITION		0.2 %	0.09 %
EXPIRATION DATE			31-MAR-2025
DATE OF MANUFACTURE			31-MAY-2022
APPEARANCE			WHITE CRYSTALLINE POWDER

Spectrum Chemical Mfg Corp  
14427 South San Pedro Street  
Gardena 90248 CA



Certificate of Analysis Results Certified by:



Himanshu Patel  
Quality Control Manager  
Spectrum Chemicals & Laboratory Products

All pharmaceutical ingredients are tested using current edition of applicable pharmacopeia.

Read and understand label and SDS before handling any chemicals. All Spectrum's chemicals are for manufacturing, processing, repacking or research purposes by experienced personnel only. It is the customer's responsibility to provide adequate hazardous material training and ensure that appropriate Personal Protective Equipment (PPE) is used before handling any chemical.

Lampiran 8. Hasil analisa ukuran partikel asam askorbat

**HORIBA**  
Scientific

HORIBA SZ-100 for Windows [Z Type] Ver2.00

**SZ-100**

033.C.PSA.VI.2022.nsz

**Measurement Results**

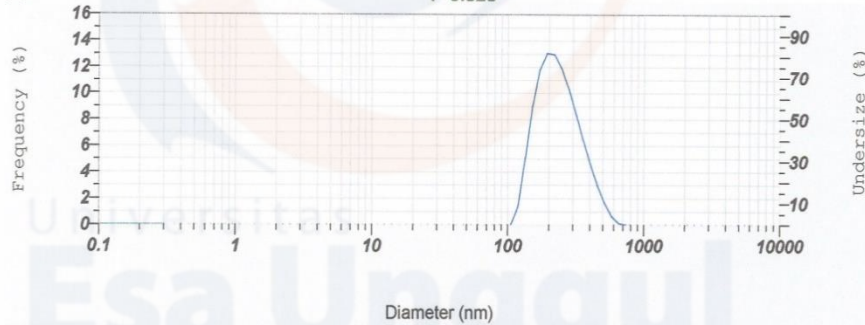
Date : Friday, June 17, 2022 11:04:22 AM  
 Measurement Type : Particle Size  
 Sample Name : AuNPs Vitamin C  
 Scattering Angle : 90  
 Temperature of the Holder : 25.0 °C  
 Dispersion Medium Viscosity : 0.896 mPa·s  
 Transmission Intensity before Meas. : 34131  
 Distribution Form : Standard  
 Distribution Form(Dispersity) : Monodisperse  
 Representation of Result : Scattering Light Intensity  
 Count Rate : 32 kCPS

**Calculation Results**

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	232.9 nm	86.5 nm	183.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	232.9 nm	86.5 nm	183.0 nm

**Cumulant Operations**

Z-Average : 254.9 nm  
 PI : 0.325



No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	22	4.40	0.000	0.000	43	57.09	0.000	0.000
2	0.38	0.000	0.000	23	4.97	0.000	0.000	44	64.50	0.000	0.000
3	0.43	0.000	0.000	24	5.61	0.000	0.000	45	72.87	0.000	0.000
4	0.49	0.000	0.000	25	6.34	0.000	0.000	46	82.33	0.000	0.000
5	0.55	0.000	0.000	26	7.17	0.000	0.000	47	93.02	0.000	0.000
6	0.62	0.000	0.000	27	8.10	0.000	0.000	48	105.10	0.000	0.000
7	0.70	0.000	0.000	28	9.15	0.000	0.000	49	118.74	1.320	1.320
8	0.80	0.000	0.000	29	10.34	0.000	0.000	50	134.16	4.963	6.313
9	0.90	0.000	0.000	30	11.68	0.000	0.000	51	151.57	9.208	15.318
10	1.02	0.000	0.000	31	13.20	0.000	0.000	52	171.25	11.798	27.118
11	1.15	0.000	0.000	32	14.91	0.000	0.000	53	193.48	13.038	40.155
12	1.30	0.000	0.000	33	16.84	0.000	0.000	54	218.60	12.950	53.105
13	1.47	0.000	0.000	34	19.03	0.000	0.000	55	248.88	11.911	65.015
14	1.68	0.000	0.000	35	21.50	0.000	0.000	56	279.04	10.291	75.308
15	1.87	0.000	0.000	36	24.29	0.000	0.000	57	315.27	8.383	83.700
16	2.11	0.000	0.000	37	27.45	0.000	0.000	58	356.20	6.444	90.143
17	2.39	0.000	0.000	38	31.01	0.000	0.000	59	402.44	4.587	94.740
18	2.70	0.000	0.000	39	35.03	0.000	0.000	60	454.89	2.957	97.696
19	3.05	0.000	0.000	40	39.59	0.000	0.000	61	513.71	1.800	99.296
20	3.45	0.000	0.000	41	44.72	0.000	0.000	62	580.41	0.909	99.905
21	3.89	0.000	0.000	42	50.53	0.000	0.000	63	655.76	0.095	100.000
								64	740.89	0.000	100.000
								65	837.07	0.000	100.000
								66	945.74	0.000	100.000
								67	1068.52	0.000	100.000
								68	1207.24	0.000	100.000
								69	1363.97	0.000	100.000
								70	1541.04	0.000	100.000
								71	1741.10	0.000	100.000
								72	1967.14	0.000	100.000
								73	2222.51	0.000	100.000
								74	2511.05	0.000	100.000
								75	2837.04	0.000	100.000
								76	3205.35	0.000	100.000
								77	3611.48	0.000	100.000
								78	4058.83	0.000	100.000
								79	4622.81	0.000	100.000
								80	5222.96	0.000	100.000
								81	5861.02	0.000	100.000
								82	6637.10	0.000	100.000
								83	7532.85	0.000	100.000
								84	8510.56	0.000	100.000

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**HORIBA**



**Lampiran 9. Hasil potensial zeta nanopartikel emas**

2022.06.21 09:27:08

**HORIBA**  
Scientific

HORIBA SZ-100 for Windows [Z Type] Ver2.00

**SZ-100**

**Measurement Results**

Zeta 033.C.PSA.VI.2022.nzt

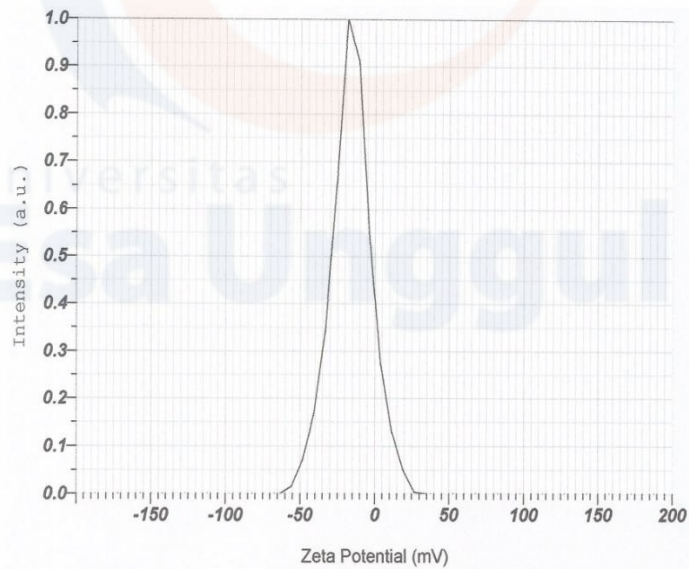
**Measurement Results**

Date : Tuesday, June 21, 2022 9:24:41 AM  
 Measurement Type : Zeta Potential  
 Sample Name : SNEDDS Vitamin C  
 Temperature of the Holder : 24.9 °C  
 Dispersion Medium Viscosity : 0.897 mPa·s  
 Conductivity : 0.130 mS/cm  
 Electrode Voltage : 3.3 V

**Calculation Results**

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-15.8 mV	-0.000122 cm <sup>2</sup> /Vs
2	-- mV	-- cm <sup>2</sup> /Vs
3	-- mV	-- cm <sup>2</sup> /Vs

Zeta Potential (Mean) : -15.8 mV  
 Electrophoretic Mobility Mean : -0.000122 cm<sup>2</sup>/Vs



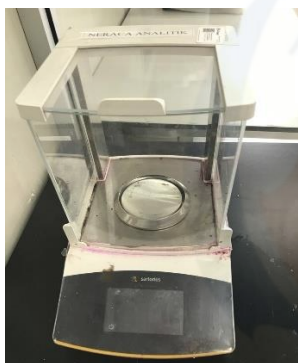
Lampiran 10. Alat dan Bahan



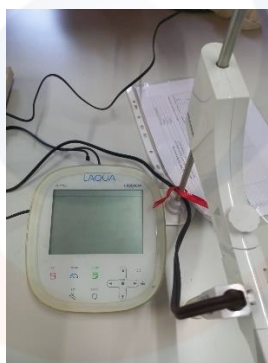
Spektrofotometer UV-Vis



Hot plate



Neraca analitik



pH meter



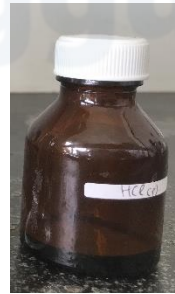
Mikropipet



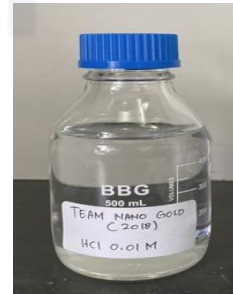
**Au Foil**



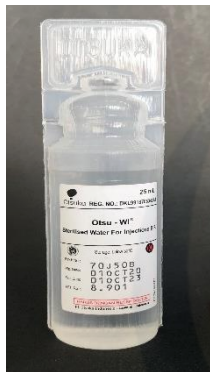
**HNO<sub>3</sub>**



**HCl**



**HCl 0,01 M**



**Aqua pro injection**



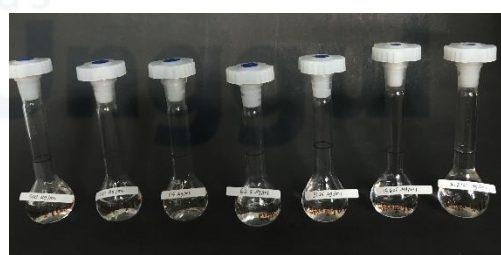
**NaOH**



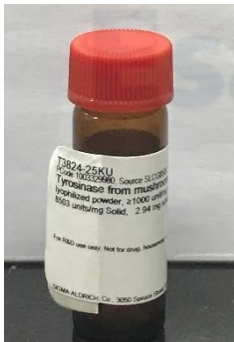
**KH<sub>2</sub>PO<sub>4</sub>**



**Dapar fosfat**



**Asam kojat**



**Enzim  
tirosinase**



**L-Tirosin**

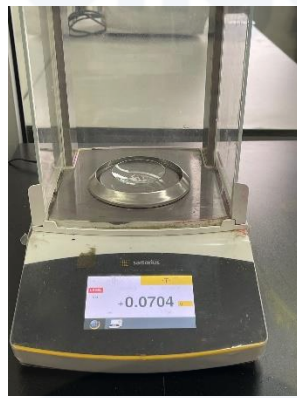


**Asam  
askorbat**

Lampiran 11. Penimbangan bahan



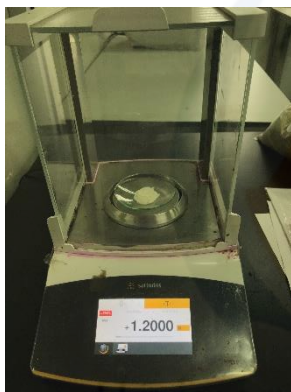
Asam askorbat 2 mM



Asam askorbat 4 mM



Asam askorbat 6 mM



Gom arab



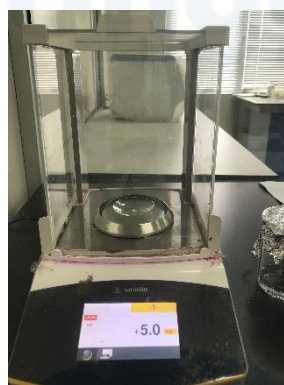
Au foil



NaOH



$\text{KH}_2\text{PO}_4$

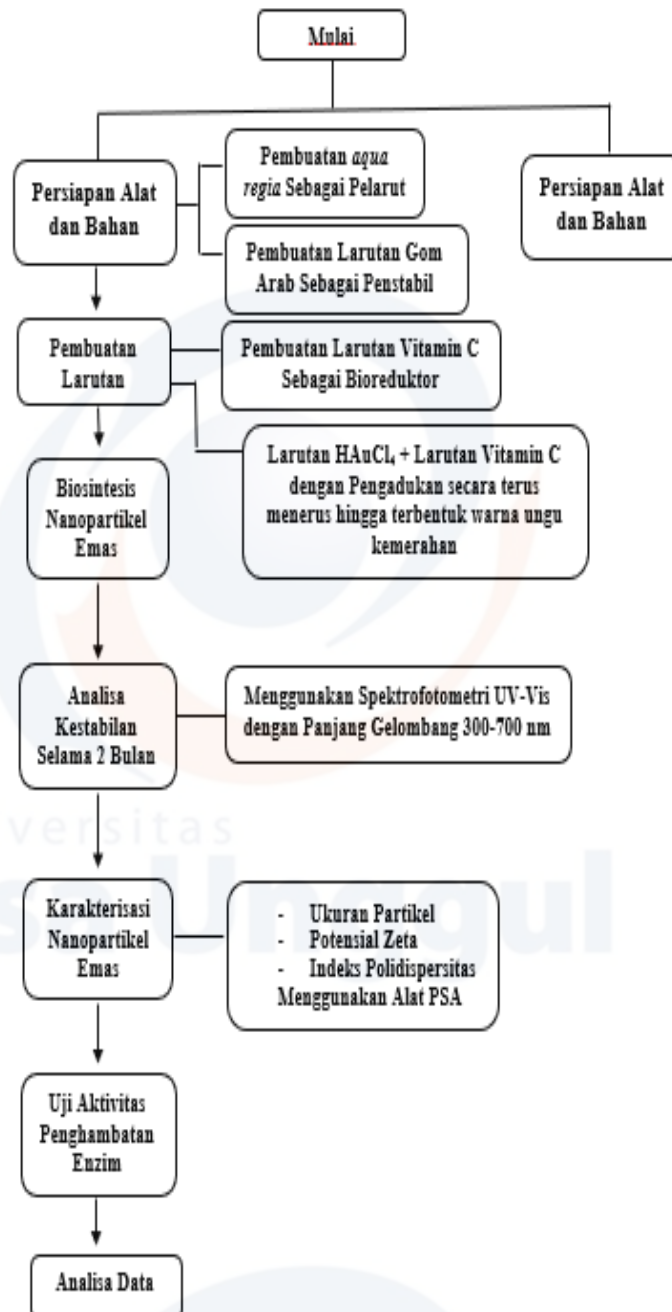


Asam kojat



L-Tirosin

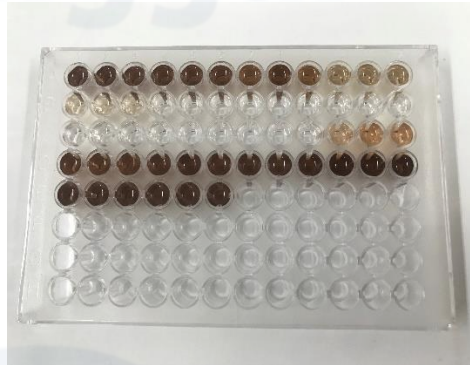
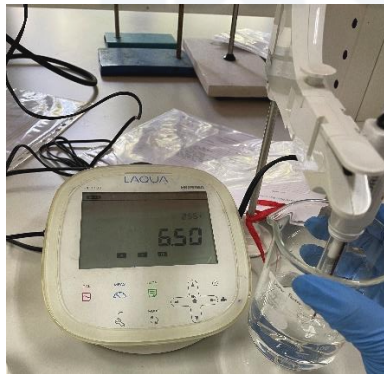
## Lampiran 12. Skema penelitian



	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

- A1-A3 : Blanko  
A4-B12 : Larutan standar asam kojat  
C1-E6 : Larutan sampel

Lampiran 14. Dokumentasi





**Lampiran 15. Hasil uji penghambatan enzim tirosinase asam kojat**

Sampel	Konsentrasi (ppm)	% Inhibisi	R <sup>2</sup>	IC <sub>50</sub>
Asam Kojat	7,81	19,47	0,9869	24,30
	15,62	32,55		
	31,25	57,04		
	62,5	82,07		
	125	95,34		

**Lampiran 16. Uji penghambatan enzim tirosinase asam askorbat**

Sampel	Konsentrasi (ppm)	% Inhibisi	R <sup>2</sup>	IC <sub>50m</sub> (ppm)
F1	11,75	16,15	0,9869	42,48
	23,5	20,44		
	47	56,96		
	94	56,96		
	188	96,56		

**Lampiran 17. Perhitungan larutan asam askorbat**

$$\begin{aligned} 2 \text{ mM} &= g = \frac{M \times Mr \times V}{1000 \text{ ml}} \\ &= g = \frac{0,002 \text{ M} \times 176,13 \times 100 \text{ ml}}{1000 \text{ ml}} \\ &= 0,0352 \text{ gram} \end{aligned}$$

$$\begin{aligned} 4 \text{ mM} &= g = \frac{M \times Mr \times V}{1000 \text{ ml}} \\ &= g = \frac{0,004 \text{ M} \times 176,13 \times 100 \text{ ml}}{1000 \text{ ml}} \\ &= 0,0704 \text{ gram} \end{aligned}$$

$$\begin{aligned} 6 \text{ mM} &= g = \frac{M \times Mr \times V}{1000 \text{ ml}} \\ &= g = \frac{0,006 \text{ M} \times 176,13 \times 100 \text{ ml}}{1000 \text{ ml}} \\ &= 0,1056 \text{ gram} \end{aligned}$$

**Lampiran 18. Perhitungan larutan substrat L-Tirosin 2 mM**

Konsentrasi substrat L-Tirosin 2 mM

$$M = \frac{\text{gram}}{BM} \times \frac{1000 \text{ ml}}{V}$$

Diketahui :

M : Molaritas (2 mM)

BM substrat L-Tirosin : 181,19

V : volume yang diinginkan (10 ml)

$$\begin{aligned} \text{Bobot substrat L-Tirosin yang harus ditimbang} &= \frac{0,002 \text{ M} \times 181,19 \times 10 \text{ ml}}{1000 \text{ ml}} \\ &= 0,0036 \text{ gram} \end{aligned}$$

**Lampiran 19. Perhitungan larutan asam kojat**

1. Pembuatan larutan induk asam kojat 500 µg/ml

Diketahui :

Konsentrasi larutan induk asam kojat yang akan dibuat : 500 µg/ml

Volume larutan induk yang akan dibuat : 10 ml

Perhitungan bobot asam kojat yang ditimbang :

$$\frac{500 \mu\text{g}}{1000 \text{ ml}} \times 10 \text{ ml} = 5 \text{ mg}$$

2. Pembuatan variasi konsentrasi larutan asam kojat 250; 125; 62,5; 31,25; 15,625; dan 7,8125 µg/ml dalam 10 ml

a.  $250 \mu\text{g/ml} = \frac{250 \mu\text{g/ml}}{500 \mu\text{g/ml}} \times 10 \text{ ml} = 5 \text{ ml}$  (diambil dari konsentrasi 500 µg/ml)

b.  $125 \mu\text{g/ml} = \frac{125 \mu\text{g/ml}}{250 \mu\text{g/ml}} \times 10 \text{ ml} = 5 \text{ ml}$  (diambil dari konsentrasi 250 µg/ml)

c.  $62,5 \mu\text{g/ml} = \frac{62,5 \mu\text{g/ml}}{125 \mu\text{g/ml}} \times 10 \text{ ml} = 5 \text{ ml}$  (diambil dari konsentrasi 125 µg/ml)

d.  $31,25 \mu\text{g/ml} = \frac{31,25 \mu\text{g/ml}}{62,5 \mu\text{g/ml}} \times 10 \text{ ml} = 5 \text{ ml}$  (diambil dari konsentrasi 62,5 µg/ml)

e.  $15,625 \mu\text{g/ml} = \frac{15,625 \mu\text{g/ml}}{31,25 \mu\text{g/ml}} \times 10 \text{ ml} = 5 \text{ ml}$  (diambil dari konsentrasi 31,25 µg/ml)

f.  $7,8125 \mu\text{g/ml} = \frac{7,8125 \mu\text{g/ml}}{15,625 \mu\text{g/ml}} \times 10 \text{ ml} = 5 \text{ ml}$  (diambil dari konsentrasi 15,625 µg/ml)

**Lampiran 20. Perhitungan larutan enzim tirosinase 333 U/mL**

- a. Pembuatan larutan induk enzim tyrosinase  
Diketahui  
Aktivitas enzim dalam sediaan 25 mg (25000 U/mL)  
Dilutkan dengan 12,5 ml Dapar fosfat = 2000 U/mL
  
- b. Pembuatan larutan enzim tirosinase 333 U/mL  
Larutan enzim tirosinase 2000 U/mL diambil sebanyak 1,665 ml  
1,665 larutan enzim tirosinase ditambahkan 10 ml dapar fosfat = 333 U/mL