3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Tyrosine Colorimetric Assay Kit

Catalog Number **MAK219**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Tyrosine (Tyr) is an aromatic amino acid that is a precursor of several important substrates such as catecholamine, thyroid hormone, and melanin pigment. A deficiency in enzymes such as homogentisic acid oxidase or phenylalanine 4-hydroxylase that degrade tyrosine result in rare, hereditary metabolic disorders.[1,2,3]

The Tyrosine Colorimetric Assay Kit provides a simple assay for measuring tyrosine in biological samples (ranging from 5–75 nmole/well). Tyrosine is determined by measuring a colorimetric product with absorbance at 492 nm (A_{492}) proportional to the amount of tyrosine present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Tyr Assay Buffer Catalog Number MAK219A	25 mL
Tyr Enzyme Mix Catalog Number MAK219B	1 vl
Tyr Standard Catalog Number MAK219C	1 vl

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter (optional for protein-containing samples)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Tyr Assay Buffer – Store the buffer at –20 °C or 2–8 °C. Allow buffer to come to room temperature before use.

Tyr Enzyme Mix – Reconstitute with 220 µL of Tyr Assay Buffer. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Tyr Standard – Reconstitute with 100 µL of water to generate a 100 mM Tyr Standard Solution. Store at 2–8 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

<u>Tyr Standards</u>

Dilute 25 µL of the 100 mM Tyr Standard with 975 µL of water and mix well to make a 2.5 mM (2.5 nmole/µL) Tyr Standard Solution. Add 0, 2, 6, 12, 18, 24, and 30 µL of the 2.5 mM (2.5 nmole/µL) Tyr Standard Solution into a 96 well plate generating 0 (blank), 5, 15, 30, 45, 60, and 75 nmole/well standards. Add water to each well to bring the volume to 150 µL.

Sample Preparation

Proteins in samples may impact background levels. To remove proteins from samples, deproteinize using a 10 kDa MWCO spin filter.

Add 80–135 µL of the samples into duplicate wells. Bring samples to a final volume of 150 µL using water.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the Tyr Enzyme Mix. The Sample Blank readings can then be subtracted from the sample readings.

Spiking duplicate sample wells with a known amount of Tyr Standard Solution (30 nmole/well) is recommended for the accurate determination of tyrosine in samples that contain endogenous compounds that might interfere with the reaction.

Assay Reaction

1. Set up the appropriate Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	**Standards and samples**	**Sample Blank**
Tyr Assay Buffer	48 µL	50 µL
Tyr Enzyme Mix	2 µL	–

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 60 minutes at room temperature. Protect plate from light during incubation.

4. Measure the absorbance (A_{492}) in a microplate reader.

Results

Calculations

Correct for the background by subtracting the measurement obtained for the 0 (blank) Tyr standard from that of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Tyr Standards to plot a standard curve. Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank value from the Sample value to obtain the corrected measurement. Using the corrected measurement, determine the amount of Tyr (nmole/well) generated by the assay (S_a).

Concentration of Tyr

$C = S_a/S_v$

where:
S_a = Amount of Tyr in unknown sample well (nmole) from standard curve
S_v = Sample volume (μL) added into the well
C = Concentration of Tyr in sample (nmole/μL)

Sample Calculation

Amount of Tyr (S_a) = 25.84 nmole (from standard curve)

Sample volume (S_v) = 10 μL

Concentration of tyrosine in sample:

25.84 nmole/10 μL = 2.584 nmole/μL

Molecular weight of tyrosine: 181.2 g/mole

2.584 nmole/μL × 181.2 ng/nmole = 468.2 ng/μL

Concentration of Tyr in spiked samples

For spiked samples, calculate the amount of Tyr in the sample wells after correcting for the Sample Blank and background.

$$C = \frac{S_p \times A_s}{(A_{sp} - A_s) \times S_v}$$

where:
S_p = Known amount of Tyr Standard spiked in well (nmole)
A_s = Corrected sample reading (A_{492}) (unspiked well)
A_{sp} = Corrected sample + spike reading (A_{492})
S_v = Sample volume (μL) added into the well
C = Concentration of Tyr in sample (nmole/μL)

Sample Calculation

Amount of Tyr Standard spike (S_p) = 30 nmole

Sample volume (S_v) = 10 μL

Corrected sample reading (A_s) = 0.599 (A_{492})

Corrected spike + sample reading (A_{sp}) = 0.702 (A_{492})

Concentration of Tyr in sample:

$$\text{nmole}/\mu\text{L} = \frac{30 \text{ nmole spike} \times 0.599}{[(0.702 - 0.599) \times 10\ \mu\text{L}]} = 17.4$$

References

1. Medes, G., A new error of tyrosine metabolism: tyrosinosis. The intermediary metabolism of tyrosine and phenylalanine. Biochem. J., **26**, 917–940 (1932).
2. Levine, R.J., and Conn, H.O., Tyrosine metabolism in patients with liver disease. J. Clin. Invest., **46**, 2012–2020 (1967).
3. La Du, B.N. et al., The nature of the defect in tyrosine metabolism in alcaptonuria. J. Biol. Chem., **230**, 251–260 (1958).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

GD,LS,KVG,MAM 10/14–1