SIGMA-ALDRICH®

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Product Information

Tyrosinase Inhibitor Screening Kit (Colorimetric)

Catalog Number **MAK257**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Tyrosinase or polyphenol oxidase (EC 1.14.18.1) is an oxidoreductase that participates in the biosynthesis of melanin, a ubiquitous biological pigment found in hair, eyes, skin, etc. Inhibition of tyrosinase has been a long-time target in the skin health research, cosmetics, and agricultural industries because of its role in browning reactions in skin pigmentation, and during fruit harvesting and handling. Skin whitening and bleaching products utilize natural or synthetic tyrosinase inhibitors in order to lighten the skin color. Polyphenols, benzaldehyde derivatives, long-chain lipids, steroids, and natural compounds have been used as tyrosinase inhibitors.

Tyrosinase catalyzes the oxidation of tyrosine, producing a chromophore that can be detected at 510 nm. In the presence of kojic acid, a reversible inhibitor of tyrosinase, the rate of oxidation of the substrate is decreased. The kit provides a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of tyrosinase inhibitors. The assay is also adaptable to a 384 well format.

Components

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

Tyrosinase Assay Buffer Catalog Number MAK257A	25 mL
Tyrosinase Substrate Catalog Number MAK257B	1 vial
Tyrosinase Catalog Number MAK257C	1 vial
Tyrosinase Enhancer Catalog Number MAK257D	0.5 mL
Inhibitor Control (Kojic Acid) Catalog Number MAK257E	1 vial

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom clear plate
- Spectrophotometric multiwell plate reader

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 small vials prior to opening. Use ultrapure water for the preparation of reagents.

Tyrosinase Substrate – Dissolve the lyophilized tyrosinase substrate in 220 µL of water. Use within two months. Keep on ice while in use.

Tyrosinase – Dissolve the lyophilized tyrosinase in 220 µL of Tyrosinase Assay Buffer. Aliquot and store at –20 °C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.

Tyrosinase Enhancer – Ready to use. Protect from light. Keep at room temperature while in use.

Inhibitor Control (Kojic Acid) – Add 75 µL of water to make a 10 mM Stock Solution of Kojic Acid. Mix well. Make a 0.75 mM Working Solution of Kojic Acid by adding 92.5 µL of water to 7.5 µL of the prepared 10 mM Kojic Acid Stock solution. Use within two months.

Storage/Stability

Store the kit at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Procedure
Read entire protocol before performing the assay.

Screening compounds, Inhibitor control and Blank Control Preparations
Dissolve test inhibitors into the proper solvent. Dilute to 5× the desired test concentration with Tyrosinase Assay Buffer before use. Add 20 µL of diluted test inhibitors, Inhibitor Control working solution, or Tyrosinase Assay Buffer into wells assigned as test inhibitors (Sample, S), Inhibitor Control (IC), or Tyrosinase Enzyme Control (EC) wells, respectively. Additional wells with serial dilutions of the test inhibitors may be prepared at this time if desired, containing 20 µL in each candidate well.

Note: Preferred final solvent concentration should not be more than 5% by volume. If solvent exceeds 5% include a Solvent Control to test the effect of the solvent on enzyme activity.

Tyrosinase Enzyme Solution Preparation
For each well, prepare 50 µL of Tyrosinase Enzyme Solution, see Table 1.

Table 1.
Preparation of Tyrosinase Enzyme Solution

Reagent	Volume
Tyrosinase Assay Buffer	48 µL
Tyrosinase	2 µL

Mix well and add 50 µL/well into wells containing test inhibitors, Inhibitor Control, and Enzyme Control. Mix. Incubate for 10 minutes at 25 °C.

Tyrosinase Substrate Solution Preparation
For each well, prepare 30 µL of Tyrosinase Substrate Solution, see Table 2.

Table 2.
Preparation of Tyrosinase Substrate Solution

Reagent	Volume
Tyrosinase Assay Buffer	23 µL
Tyrosinase Substrate	2 µL
Tyrosinase Enhancer	5 µL

Mix and add 30 µL of Tyrosinase Substrate Solution into each well. Mix well.

Measurement
Measure the absorbance in kinetic mode for 30–60 minutes at 510 nm. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the absorbance (Abs_1 and Abs_2).

Results
Calculations
Calculate the slope for all samples, including Enzyme Activity Control (EC), by dividing the net ΔAbs (Abs_2–Abs_1) values by the time ΔT (T_2–T_1). Calculate % Relative Inhibition as follows:

$$\% \text{ Relative Inhibition} = [\text{Slope(EC)} - \text{Slope(S)}]/\text{Slope(EC)} \times 100$$

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	Flat-bottom clear plates are preferred for this assay.
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 samples will be used 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 Mix 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 mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix 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

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