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Tyrosinase Inhibitor Screening Kit (Colorimetric)

(Catalog # K575-100; 100 assays; Store at -20°C) *9/14*

I. Introduction:

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.

II. Application:

Screening/characterizing tyrosinase inhibitors

III. Kit Contents:

Components	K575-100	Cap Code	Part Number
Tyrosinase Assay Buffer	25 ml	WM	K575-100-1
Tyrosinase Substrate	1 vial	Red	K575-100-2
Tyrosinase (Lyophilized)	1 vial	Blue	K575-100-3
Tyrosinase Enhancer	0.5 ml	Purple	K575-100-4
Inhibitor Control (Kojic Acid)	1 vial	Orange	K575-100-5

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Microplate reader

V. Storage and Handling:

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VI. Reagent Preparation & Storage

- **Tyrosinase Substrate:** Dissolve the lyophilized tyrosinase substrate in 220 µl ddH_2O. Use within two months. Keep on ice while in use.
- **Tyrosinase:** Dissolve the lyophilized tyrosinase in 220 µl 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 ddH_2O to make a stock solution of 10 mM Kojic Acid. Mix well. Make a 0.75 mM working solution of Kojic Acid by adding 92.5 µl of ddH_2O to 7.5 µl of 10 mM Kojic Acid Stock solution. Use within two months.

VII. Tyrosinase Inhibitor Screening Protocol:

1. **Screening compounds, Inhibitor control and Blank Control Preparations:** Dissolve test inhibitors into proper solvent. Dilute to 5X the desired test concentration with Tyrosinase Assay Buffer before use. Add 20 µl 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.

2. **Tyrosinase Enzyme Solution Preparation:** For each well, prepare 50 µl Tyrosinase Enzyme Solution.

   48 µl Tyrosinase Assay Buffer
   2 µl Tyrosinase

   Mix well & add 50 µl/well into wells containing test inhibitors, Inhibitor Control & Enzyme Control. Mix. Incubate for 10 min. at 25ºC.

3. **Tyrosinase Substrate solution preparation:** For each well, prepare 30 µl of Tyrosinase Substrate solution

   23 µl Tyrosinase Assay Buffer
   2 µl Tyrosinase Substrate
   5 µl Tyrosinase Enhancer

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

4. **Measurement**: Measure the absorbance in kinetic mode for 30-60 min. at 510 nm. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the Absorbance (Abs_1 and Abs_2).

5. **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:

$$\% \textbf{ Relative Inhibition} = \frac{(\textbf{Slope of EC} - \textbf{Slope of S})}{\textbf{Slope of EC}} \textbf{ x 100}$$

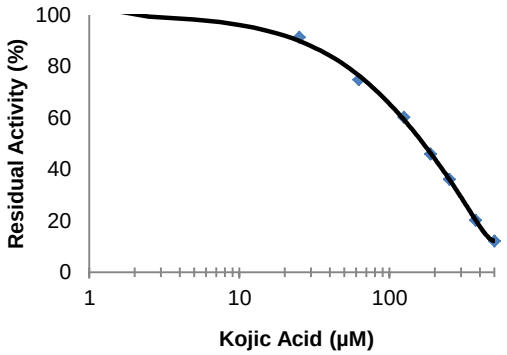

Figure: Inhibition of Tyrosinase Enzymatic Activity with Kojic Acid. Assay was performed following kit protocol.

VIII. RELATED PRODUCTS:

Asparaginase Activity Colorimetric/Fluorometric Assay Kit (K754)
Aspartate Aminotransferase Activity Colorimetric Assay Kit (K753)
Glutamate Dehydrogenase Activity Colorimetric Assay Kit (K729)
Arginase Activity Colorimetric Assay Kit (K755)

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