Tyrosinase Inhibitor Assay Kit

Catalog Number: EZ2019-01

For Research Use Only. Not for use in Diagnostic Procedures.

1. Introduction

Tyrosinase Inhibitor Screening Kit (Colorimetric) provides a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of tyrosinase inhibitors. Tyrosinase catalyzes the oxidation of tyrosine, producing a chromophore that can be detected at OD = 520 nm. In the presence of Benzoic Acid, the rate of oxidation of the substrate is decreased. Tyrosinase or polyphenol oxidase 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.

- Highly Sensitive Assay to Screen for tyrosinase inhibitors
- Detection range of 10ppm to 1,500ppm Benzoic Acid
- Highly reproducibility
- Stable formulation of ready to use Reaction Facilitator (tyrosinase)

The kit provides a rapid, simple, sensitive, and reliable test suitable for screening of tyrosinase inhibitors.

2. Contents (96 determinations)

Component Name	Volumes	Storage
Reaction Facilitator	200 µL	-15 to -25°C
Chromophore	1.5 ml x 2	2 - 8°C
Reaction Buffer 1	18 mL	RT
Reaction Buffer 2	2.6 mL	RT
Substrate [S]	700 µL	2 - 8°C
Inhibitor Control (Benzoic Acid 15,000ppm)	0.5 mL	2 - 8°C
96 well plate	1 each	RT

***Required materials not included in kit**

- Microplate reader (520nm)
- Vortex mixer
- 10, 20, 100 and 1000ul pipettes
- 100% methanol (HPLC grade)

3. Tyrosinase Inhibitor Test Method

- The Tyrosinase Inhibitor Assay kit is designed specifically to screen for tyrosinase inhibitors in samples or compound libraries. In the presence of a tyrosinase inhibitor, the rate of chromophore production is reduced in a concentration dependent fashion. The higher the concentration of Inhibitor the less color is produced, the kit enables for qualitative or quantitative determination of concentration.

- The tyrosinase inhibition can be measured by reading the absorbance of the reactions at 520nm, generating a standard curve using the supplied inhibitor control included in the kit and quantifying unknown sample concentrations using linear regression analysis.

Suggested Measuring range	Number of determinations
50 – 100 – 200 – 500 – 1,500 ppm Benzoic Acid	96

4. Instructions

Note: Perform the reaction by mixing the following components in the specific order described below into one well of the provided 96 well plate for each sample, positive and negative control (use a new pipet for each step and for each well).

- Step 1. 167µL of Reaction Buffer 1
- Step 2. 31µL of Chromophore
- Step 3. 2µL of Reaction Facilitator
- Step 4. 25µL of Reaction Buffer 2

- Step 5. 40µL of sample or standards **NOTE:** Methanol concentration should be less than 15% by volume. If Methanol exceeds 15% OR is using a different solvent, include solvent controls to test the effect on the solvent on enzyme activity.
- Step 6. 7µL of Substrate [S]
- Step 7. Mix the components in the well by pipetting up and down 3-4 times
- Step 8. Incubate at room temperature for 25 minutes
- Step 9. Read the plate by measuring absorbance at 520nm.

Component	Volume per well
Reaction Buffer 1	167µL
Chromophore	31µL
Reaction Facilitator	2µL
Reaction Buffer 2	25µL
Sample	40µL
Substrate	7µL

5. Master Mix Method

Using a master mix is an acceptable approach to performing the preparation of reagents (ensure overage of 10% to account for pipetting efficiency.

For example, to make a master mix for 15 reactions perform the following in a tube:

- 2.505mL of Reaction Buffer 1
- 465µL of Chromophore
- 30µL of Reaction Facilitator
- 375ul of Reaction Buffer 2
- Mix well

Set up reaction in 96 well plate from the master mix by:

- Step 1. Aliquoting 225µL of the master mix into each well of the 96 well plate.
- Step 2. Add 40µL of sample or standard

- Step 3. Add 7µL of Substrate [S]
- Step 4. Mix the components in the well by pipetting up and down 3-4 times
- Step 5. Incubate at room temperature for 25 minutes.
- Step 6. Read the plate by measuring absorbance at 520nm.

6. Method Control

It is best to run standards with each unknown sample set to ensure comparable readings from the day, time, and user. If quantitative results are required, make sure to perform a series of standard curve reactions which can be used to extrapolate the concentration in the sample being analyzed, loading into a 96 well plate and reading the samples at 520nm.

A 15,000 ppm Benzoic Acid Solution is included in the kit can be used to produce sample spiked controls or a set of standards in a solvent as needed.

7. Sample Preparation

- Prepare test compounds by diluting into 100% methanol (or desired solvent) before use. Use 40µL of test inhibitor per well, preferably in duplicate or triplicate.
- Prepare inhibitor Control wells by diluting the 15,000ppm stock into 100% methanol to a concentration range 50-1,500ppm before use. Example: to make 1mL of a 1,500ppm inhibitor control add 100ul of the inhibitor control (benzoic acid 15,000ppm) into 900ul of 100% methanol.
- Enzyme Control wells (EC) = 40µL 100% Methanol.
- If using a solvent other than methanol, include a solvent control (SC) in a reaction well= 40µL alternative solvent.

Concentration Calculation:

Standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ppm on a logarithmic curve. Relative absorbance (%) = absorbance standard (or sample) x 100 absorbance zero standard

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested compound from the standard curve.

The following figure is a typical standard curve produced using benzoic acid.

Example of standard curve:

Well Contents	OD at 520nm	Bo% (relative absorbance %
0ppm	0.858	100
50ppm	0.687	80%
100ppm	0.572	67%
200ppm	0.401	47%
500ppm	0.197	23%
1,500ppm	0.135	16%

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

Contact Us
3913 Todd Lane, Suite 310
Phone: 512- 333-1330
Email: sales@attogene.com
Web: www.attogene.com
EZ2019-01.V6