AttoTector Tyrosinase Inhibition Assay
Catalog Number: EZ2019-01

Kit Contents:

Component	Amount	Storage Condition
Tyrosinase Assay Buffer	25 mL	2 - 8°C
Tyrosinase Substrate	220 µL	2 - 8°C
Tyrosinase	220 µL	2 - 8°C
Tyrosinase Enhancer	500 µL	2 - 8°C
Inhibitor Control (1,500ppm Benzoic Acid)	50 µL	2 - 8°C

Tyrosinase Test Method:

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 = 510 nm. In the presence of kojic Acid, a reversible inhibitor of tyrosinase, 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.

Required Materials not provided:

- Micro-pipettes with disposable plastic tips (25-1000 µL)
- Multi-channel pipette (50-250 µL) or stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips
- Timer
- Microtiter plate reader (wavelength 405-450 nm)

Assay Protocol:

Method control: It is best to run a set of negative and positive controls with each sample set run to ensure comparable readings from the day, time and user. Depending on the inhibitor being detected, a spike solution that can be used to generate the positive control can be made and added into control wells.

Sample Preparation:

Dissolve test compounds into proper solvent and dilute 5x the desired test concentration with tyrosinase assay buffer before use.

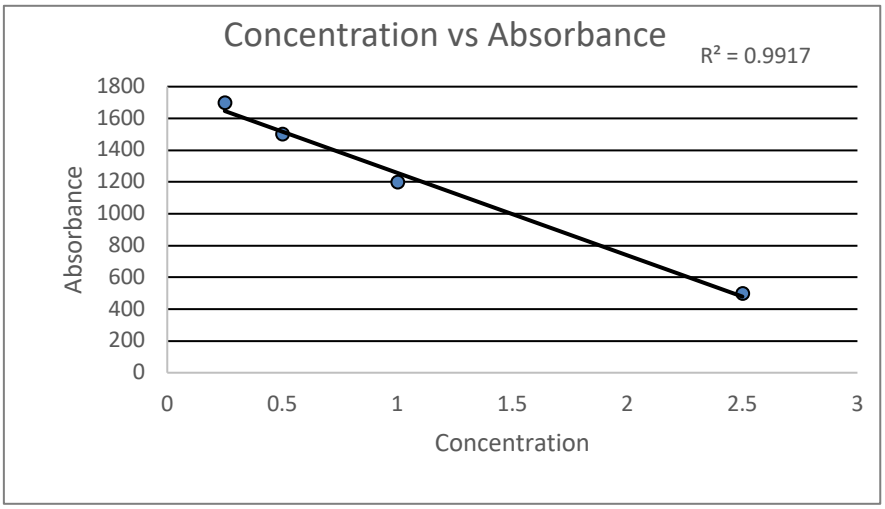
Protocol:

1. Equilibrate materials to room temperature.
2. Set up reaction wells:
   Sample wells (S) = 20µL test inhibitors.
   - Inhibitor Control wells (IC) = 20µL Tyrosinase inhibitor working solution (100ppm).
   - Enzyme Control wells (EC) = 20µL Tyrosinase Assay Buffer.
   - OPTIONAL: Solvent control (SC) = 20µL solvent.

   NOTE: preferred final solvent concentration should not be more than 5% by volume. If solvent exceeds 5%, include solvent control to test the effect on the solvent on enzyme activity.
3. Prepare 50ul of Tyrosinase Enzyme Solution for each well by adding 45ul of Tyrosinase Assay Buffer and 2ul of Tyrosinase Enzyme into well
4. Add 50µL of Tyrosinase Enzyme Solution into each well
5. Incubate at 25ºC for 10 minutes
6. Add 30ul of Tyrosinase Substrate Solution to each well.
7. To the remainder of the wells add 5ul of the test compounds.
8. Incubate for 15 minutes
9. For each reaction well mix 154ul of Assay Buffer with 1ul Substrate and 0.5ul of Chromogen. Add 150ul of this working reagent to each well.
10. Incubate the plate at room temperature; after 0, 10, 30 and 60 min.
11. Review color change by reading the absorbance at 510 nm.
12. Analyze % inhibition.

	A	B	C	D	E
1	Neg				
2	Neg				
3	Pos				
4	Pos				
5	Sample 1				
6	Sample 1				
7	Sample 2				
8	Sample 2				

If quantitative results are required, it is possible to set up a set of standards at known concentrations of specific pesticides which can be used to extrapolate the concentration in the sample being analyzed, loading into a 96 well plate and reading the samples at 510± 5 nm.



The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Acetylcholinesterase in the known or unknow inhibitors. We recommend using the Online Linear Regression.