
# Tyrosinase Activity Assay Kit

## Catalog Number: EZ2017

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

## 1. Introduction

Tyrosinase (EC 1.14.18.1) is a copper-binding enzyme that is expressed across a vast range of species ranging from bacteria and fungi to mammals. It is involved in two sequential reactions of the melanin synthesis pathway: first being the hydroxylation of a monophenol and second the conversion of an ortho-diphenol to a quinone. Quinone then undergoes a series of reactions including polymerization to form melanin. Tyrosinase is of great interest to the agriculture industry since it causes browning of fruits, vegetable and mushrooms, as well as to the cosmetic industry as it causes skin darkening. Development and screening of tyrosinase inhibitors, therefore, is very useful for conditions such as hyperpigmentation and melasma. Tyrosinase activity is significantly increased in melanoma. Therefore, the detection of tyrosinase activity could be promising as a specific diagnostic test for melanoma and may be useful in monitoring patient response to melanoma treatments. This Tyrosinase Activity Assay Kit is a simple one-step, plate-based assay for the measurement of tyrosinase activity in biological samples. In this assay, tyrosinase catalyzes the conversion of a phenolic substrate to a Quinone intermediate, which reacts with the tyrosine enhancer forming a highly stable chromophore with absorbance at 520 nm. The assay can detect as low as 30 μU Tyrosinase in biological samples.

- Highly Sensitive Assay to Screen for Tyrosinase Activity
- 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 concentration in samples.**

## 2. Contents (96 determinations)

| Component Name | Volumes | Storage |
|---|---|---|
| Tyrosinase Positive Control (166, 16.6, 1.66, 0.166 Units/uL) | 50 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 |
| Tyrosinase Lysis Buffer | 5mL | RT |
| 96 well plate | 1 each | RT |

**Required materials not included in kit**

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

## 3. Tyrosinase Test Method

The Tyrosinase Activity Assay kit is designed specifically to screen for Tyrosinase in samples. The ability to detect as low as 30 μU Tyrosinase in a sample.

The Tyrosinase concentration can be measured by reading the absorbance of the reactions at 520nm, generating a standard curve using standards and quantifying unknown sample concentrations using linear regression analysis.

## 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 Sample, Tyrosinase Lysis Buffer or Tyrosinase Positive Control
- Step 4. 25μL of Reaction Buffer 2
- 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 Buffer 2 | 25µL |
| Sample or Tyrosinase Positive Control | 2µL |
| 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
- 375ul of Reaction Buffer 2
- Mix well

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

- Step 1. Aliquoting 223µL of the master mix into each well of the 96 well plate
- Step 2. Add 2µL of Sample, Tyrosinase Lysis Buffer or Tyrosinase Positive Control
- 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 duplicate 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.

Tyrosinase Positive Control Solutions included in the kit can be used to produce sample spiked controls or a set of standards in a negative extract as needed.

## 7. Sample Preparation:

Homogenize cells (8 x $10^6$ cells) or tissue (50 mg) with 500 µl ice-cold tyrosinase lysis buffer to perform lysis and keep on ice for 10 minutes followed by centrifugation at 10,000 x g for 15 minutes at 4ºC. Collect the supernatant (lysate) and estimate protein concentration using preferred method. Protein concentration should range between 1 and 2.5 µg/µl). Dilute the lysate if needed using Tyrosinase Assay Buffer. Use the samples for activity analysis immediately; if that is not possible, they may be stored at -80 ºC. Prepare two wells for each sample labeled "Sample Background Control" (SBC) and "Sample" (S). Add the same volume (2 - 25 µl, i.e., 5 – 25 µg protein) into each of these wells. **For Positive Control:** add 2 µl of the provided Tyrosinase Positive Control into the desired well. For Assay Background Control (i.e., substrate background): add Tyrosinase Assay Buffer to a well.

## Concentration Calculation:

Standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in units 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 sample from the standard curve.

## Customer Notes

## 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
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