

Version 1a updated 5 May 2021

# ab252899

## Tyrosinase Activity Assay Kit (Colorimetric)

View Kit datasheet: <https://www.abcam.com/ab252899>  
(use <https://www.abcam.cn/ab252899> for china, or  
<https://www.abcam.co.jp/ab252899> for Japan)

For the measurement of tyrosinase activity in cell and tissue lysates using a 96-well plate format.

This product is for research use only and is not intended for diagnostic use.

## Table of Contents

1. Overview	3
2. Protocol Summary	4
3. Materials Supplied and Storage	5
4. Materials Required, Not Supplied	6
5. General guidelines, precautions, and troubleshooting	7
6. Reagent Preparation	8
7. Assay Procedure	11
8. Typical Data	13
9. Notes	15

## 1. Overview

Tyrosinase Activity Assay Kit (Colorimetric) (ab252899) is a simple one-step, plate-based assay for the measurement of tyrosinase activity in biological samples. In this assay, tyrosinase catalyses the conversion of a phenolic substrate to a Quinone intermediate, which reacts with the tyrosine enhancer forming a highly stable chromophore with absorbance at 510 nm. The assay can detect as low as 30  $\mu$ U Tyrosinase in biological samples.



## 2. Protocol Summary

Prepare all samples, standards and controls as instructed.



Prepare the chromophore standard curve dilutions and plate into a 96-well plate.



Add 50  $\mu\text{L}$  of Sample Background Control mix to the appropriate wells and 50  $\mu\text{L}$  of Reaction Mix to the Sample, Substrate Background and Positive Control wells.



Read the absorbance immediately using a plate reader at 37 °C at 510 nm in kinetic mode. Standards may be read in end-point mode.



Record absorbance at 30 second intervals for 10-15 minutes.



Use the OD values to calculate the Sample Tyrosine specific activity in Units/mg.

### 3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Tyrosinase Assay Buffer	25 mL	-20°C	4°C
Tyrosinase Substrate	1.1 mL	-20°C	-20°C
Tyrosinase Enhancer	1 vial	-20°C	-20°C
Chromophore Standard (1mM)	400 µL	-20°C	-20°C
Tyrosinase Positive control	1 vial	-20°C	-20°C

## 4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-Well clear plate with flat bottom.
- Multi-well spectrophotometer.

## 5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

[www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines)

For typical data produced using the assay, please see the assay kit datasheet on our website.

## 6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 6.1 Tyrosinase Assay Buffer:

Warm to room temperature before use.

### 6.2 Tyrosinase Substrate:

Thaw on ice before use. Aliquot and store the remaining stock at -20°C in dark vials. Do not expose to light.

### 6.3 Tyrosinase Enhancer:

Reconstitute in 550  $\mu$ L water. Aliquot and store the remaining stock at -20°C.

### 6.4 Chromophore Standard:

Ready to use as supplied.

### 6.5 Tyrosinase Positive control:

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



## 7. Sample preparation

- Homogenize cells ( $8 \times 10^6$  cells) or tissue (50 mg) with 500  $\mu\text{L}$  ice-cold Tyrosinase Assay buffer to perform lysis and keep on ice for 10 minutes followed by centrifugation at  $10,000 \times g$  for 15 minutes at  $4^\circ\text{C}$ .
- Collect the supernatant (lysate) and estimate protein concentration using preferred method. We recommend BCA protein assay kit
- Protein concentration should range between 1 and  $2.5 \mu\text{g} / \mu\text{L}$ . 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  $-80^\circ\text{C}$ . Prepare two wells for each sample labeled "Sample Background Control" (SBC) and "Sample" (S). Add the same volume (2 - 25  $\mu\text{L}$ , i.e. 5 – 25  $\mu\text{g}$  protein) into each of these wells.

**For Positive Control:** add 2  $\mu\text{L}$  of the provided Tyrosinase Positive Control into the desired well. Adjust volume in each well to 50  $\mu\text{L}$  with Tyrosinase Assay Buffer.

**For Assay Background Control (i.e., substrate background):** add 50  $\mu\text{L}$  of Tyrosinase Assay Buffer to a well.

**ΔNote:** For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.

## 8. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

- 8.1 Add 0, 2, 4, 6, 8, 10  $\mu\text{L}$  of the 1 mM standard into a series of wells in a clear 96-well plate to obtain 2, 4, 6, 8 and 10 nmol / well.
- 8.2 Adjust the volume of each well to 100  $\mu\text{L}$  with Tyrosinase Assay Buffer.

### **Optional (for samples that have low activity):**

- 8.3 Dilute 10  $\mu\text{l}$  of the Chromophore Standard with 90  $\mu\text{l}$  of Tyrosinase Assay Buffer to obtain 100  $\mu\text{M}$  Chromophore Standard.
- 8.4 Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  of the diluted Chromophore Standard into a series of wells in a clear 96-well plate to obtain 0, 200, 400, 600, 800 and 1000 pmol/well of Chromophore. Bring the volume to 100  $\mu\text{l}$  with Tyrosinase Assay Buffer.

## 9. Assay Procedure

### 9.1 Reaction mix:

1. Mix enough reagents for the number of assays to be performed.
2. Add "SBC Mix" to "Sample Background Control" wells and "Reaction Mix" to Assay Background Control (substrate background), Sample, and Positive Control wells. For each well, prepare 50  $\mu\text{L}$ .

	SBC Mix	Reaction Mix
Tyrosinase Assay Buffer	45 $\mu\text{L}$	35 $\mu\text{L}$
Tyrosinase Substrate	-	10 $\mu\text{L}$
Tyrosinase Enhancer	5 $\mu\text{L}$	5 $\mu\text{L}$

3. Mix well. Add the reaction Mix to wells of the 96-well plate.
4. Do not add reaction mix to the Standard Curve wells.
5. Have the plate reader ready at 37°C at absorbance 510 nm on kinetic mode, and record every 30 seconds.

**ΔNote:** Prepare Reaction SBC Mix immediately before adding it to the wells.

### 9.2 Measurement:

1. Immediately start recording absorbance at 30 second intervals for 10-15 minutes for samples with high tyrosinase activity and for 60-90 minutes for samples with low tyrosinase activity. Standard curve may be read in end point mode.

### 9.3 Calculation:

1. Subtract sample background control OD values from sample OD values. If assay background control OD values are higher than sample background control, subtract those values from sample OD values instead.
2. Estimate the amount of chromophore formed using the standard curve.
3. Calculate  $\Delta M$ , which is the change in amount of chromophore between time  $t_1$  and  $t_2$ , such that  $t_1$  and  $t_2$  both fall in the linear portion of the reaction.
4. Tyrosinase specific activity may be calculated using the following equation:

$$\text{Tyrosinase activity} = \Delta M / (\Delta t \times P) \text{ (nmol / (min} \times \mu\text{g))} = \text{mUnits} / \mu\text{g} = \text{Units} / \text{mg}$$

Where:

$\Delta M$  = Linear change in amount of chromophore  $\Delta t$  (nmol).

$\Delta t$  =  $t_2 - t_1$  (min)

$P$  = Sample protein content added to well ( $\mu\text{g}$ )

## 10. Typical Data

Data provided for demonstration purposes only.

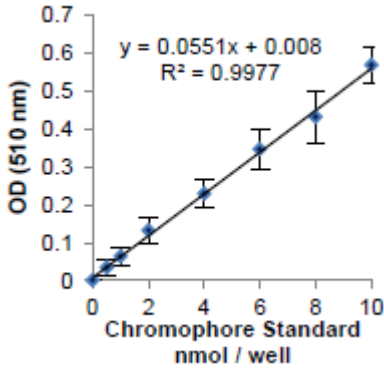


Figure 1. Chromophore Standard curve

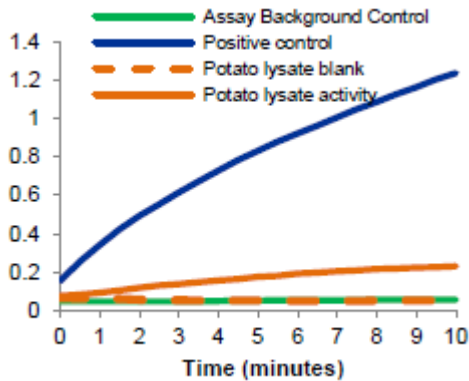
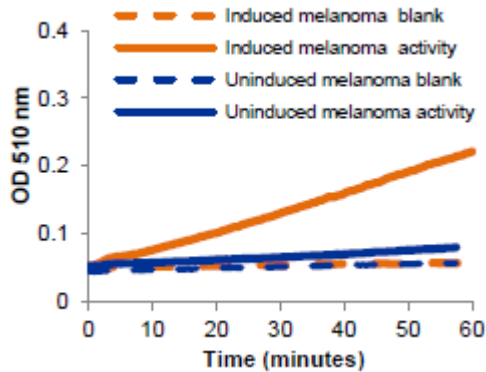
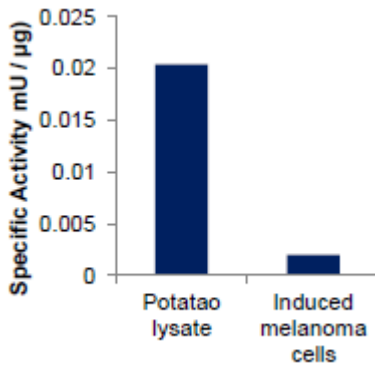


Figure 2. Enzyme kinetics for tyrosinase positive control and for potato lysate (16  $\mu$ g protein).



**Figure 3.** Enzyme kinetics for tyrosinase activity in uninduced melanoma cells cultured in EMEM + 10% FBS (15  $\mu\text{g}$  protein), and melanoma cells induced for increased tyrosinase activity by culturing for 4 days in EMEM + 0.5% FBS, supplemented with 500  $\mu\text{M}$  cAMP, 100  $\mu\text{M}$  PDE inhibitor IBMX and 100  $\mu\text{M}$   $\text{Cu}^{2+}$  (10  $\mu\text{g}$  protein).



**Figure 4.** Tyrosinase specific activity in potato lysate and melanoma cells.

## 11. Notes

## Technical Support

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