ab204715

Tyrosinase Inhibitor Screening Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate screening of potential Tyrosinase inhibitors.

View kit datasheet: www.abcam.com/ab204715
(use www.abcam.cn/ab204715 for China, or www.abcam.co.jp/ab204715 for Japan)

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 2a, Last Updated 21 August 2023

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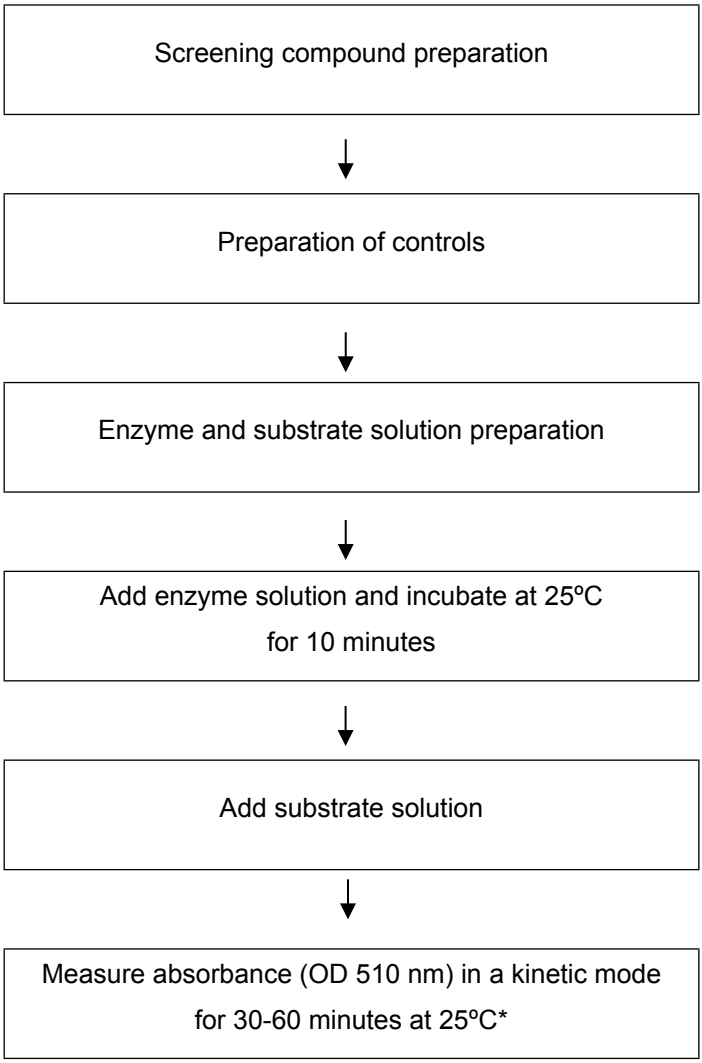
1. BACKGROUND

Tyrosinase Inhibitor Screening Kit (Colorimetric) (ab204715) 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.

The assay is also adaptable to a 384-well format.

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.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer XLII/Tyrosinase Assay Buffer	25 mL	-20°C	-20°C
Tyrosine/Tyrosinase Substrate	1 vial	-20°C	-20°C
Tyrosinase/Tyrosinase (Lyophilized)	1 vial	-20°C	-20°C
Tyrosinase Enhancer	500 µL	-20°C	-20°C
Kojic Acid/Inhibitor Control (Kojic Acid)	1 Vial	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- Inhibitor compound of choice
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips
- Microcentrifuge
- Colorimetric microplate reader – equipped with filter for OD = 510 nm
- 96 well plate: clear plate with flat bottom
- Heat block or water bath

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety regulations.
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer XLII/Tyrosinase Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Tyrosine/Tyrosinase Substrate:**

Dissolve the lyophilized Tyrosine/Tyrosinase substrate in 220 µL ddH_2O. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.

9.3 **Tyrosinase/Tyrosinase (lyophilized):**

Dissolve the lyophilized Tyrosinase in 220 µL Assay Buffer XLII/Tyrosinase Assay Buffer. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw cycles. Store at -20°C. Use within two months. Keep on ice while in use.

9.4 **Tyrosinase Enhancer:**

Ready to use as supplied. Protect from light. Aliquot enhancer so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles. Keep at room temperature while in use.

9.5 **Kojic Acid/Inhibitor Control (Kojic Acid):**

Add 75 µL of ddH_2O to make a stock solution of 10 mM Kojic Acid. Mix well. Aliquot Kojic Acid so that you have enough volume to perform the desired number of assays. Store at -20°C.

Prior to use, make a 0.75 mM working solution of Kojic Acid by mixing 92.5 µL ddH_2O + 7.5 µL Kojic Acid Stock solution. Use within two months.

10. SAMPLE PREPARATION

- Always prepare a fresh set of samples and controls for every use.

10.1 Screening Compounds:

10.1.1 Dissolve test compounds into proper solvent.

10.1.2 Dilute to 5X the desired test concentration with Assay Buffer XLII/Tyrosinase Assay Buffer before use.

NOTE: *We suggest using different volumes of testing compounds if effective concentration is unknown.*

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

11.1 **Set up reaction wells:**

- Sample wells (S) = 20 µL test inhibitors.
- Inhibitor Control wells (IC) = 20 µL Tyrosinase inhibitor working solution – Kojic Acid (0.75 mM).
- Enzyme Control wells (EC) = 20 µL Assay Buffer XLII/Tyrosinase Assay Buffer.
- OPTIONAL: Solvent control (SC) = 20 µL 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.*

11.2 **Prepare Tyrosinase Enzyme Solution:**

Prepare 50 µL of Tyrosinase Enzyme Solution for each well:

Component	Enzyme Solution (µL)
Assay Buffer XLII/Tyrosinase Assay Buffer	**48**
Tyrosinase/Tyrosinase Enzyme	**2**

Mix sufficient reagents for the number of assays to be performed. Prepare a master mix of the Enzyme Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions + 1).

11.3 Add 50 µL of Tyrosinase Enzyme Solution to each well.

11.4 Incubate at 25ºC for 10 minutes.

11.5 **Tyrosine/Tyrosinase Substrate Solution:**

Prepare 30 µL of Tyrosine/Tyrosinase Substrate Solution for each well:

Component	Substrate Solution (µL)
Assay Buffer XLII/Tyrosinase Assay Buffer	23
Tyrosine/Tyrosinase Substrate	2
Tyrosinase Enhancer	5

The table below shows the set up reaction wells:

Component	Sample Well (S) (µL)	Inhibitor Control (IC) (µL)	Enzyme control (EC) (µL)	Solvent Control (SC) (µL)
Test inhibitors	20	0	0	0
Tyrosinase Inhibitor working solution Kojic Acid	0	20	0	0
Assay Buffer XLII/Tyrosinase Assay Buffer	0	0	20	0
Solvent	0	0	0	20
Tyrosinase Enzyme Solution	50	50	50	50
Tyrosine/Tyrosinase Substrate Solution	30	30	30	30

11.2 Measure absorbance on a microplate reader at OD = 510 nm in a kinetic mode, every 2 – 3 minutes, for at least 30-60 minutes.

***NOTE**: Incubation time depends on the Tyrosinase activity in samples. Longer incubation times may be required if Tyrosinase activity is low.*

We recommend measuring the OD in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the Tyrosinase activity of the samples.

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Average the duplicate reading for each test sample compound, Kojic Acid/Inhibitor Control and Enzyme control.

12.2 Choose two time points (T1 and T2) in the linear range of the plot and obtain the corresponding values for the absorbance (A_1 and A_2).

12.3 Calculate the slope for all samples (S), Inhibition Control (IC) and Enzyme Control (EC) by dividing the net ΔA (A_2-A_1) values with the time ΔT (T_2-T_1).

12.4 Calculate the % Relative inhibitions as follows:

$$\%\ Relative\ Inhibition = \frac{Slope\ of\ EC\ -\ Slope\ of\ S}{Slope\ of\ EC} \times 100$$

NOTE:

If OD_{510nm} of SC < OD_{510nm} of EC = make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent.

If OD_{510nm} of S < OD_{510nm} of IC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. TYPICAL DATA

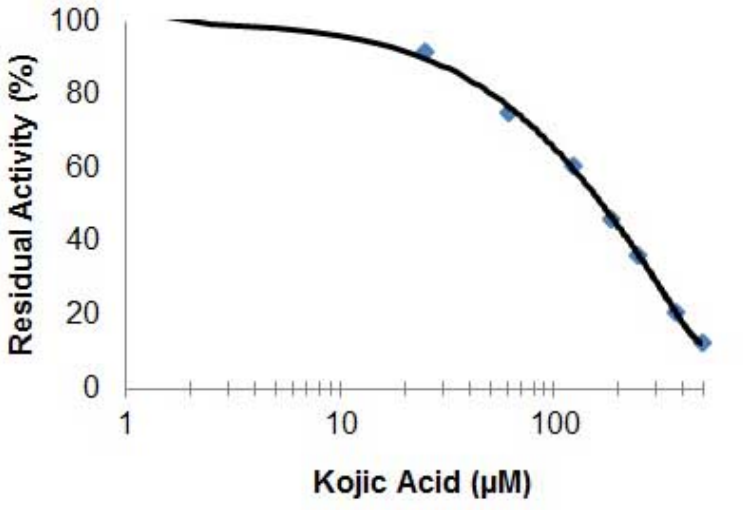


Figure 1. Inhibition of Tyrosinase Enzymatic Activity with Kojic Inhibitor. Assay was performed following kit protocol.

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare enzyme mix, substrate mix and get equipment ready.
- Prepare samples and dissolve test inhibitors in suitable solvent.
- Prepare Tyrosinase solution for all wells to be set up (50 µL/well)

Component	Enzyme Solution (µL)
Assay Buffer XLII/Tyrosinase Assay Buffer	**48**
Tyrosinase/Tyrosinase Enzyme	**2**

- Set up plate as follows:

Component	Sample Well (S) (µL)	Inhibitor Control (IC) (µL)	Enzyme Control (EC) (µL)	Solvent control (SC) (µL)
Test inhibitors	**20**	**0**	**0**	**0**
Tyrosinase Inhibitor working solution Kojic Acid	**0**	**20**	**0**	**0**
Assay Buffer XLII/Tyrosinase Assay Buffer	**0**	**0**	**20**	**0**
Solvent	**0**	**0**	**0**	**20**
Tyrosinase Enzyme Solution	**50**	**50**	**50**	**50**

- Incubate 25° C 10 min.
- Prepare 30 µL of Tyrosine/Tyrosinase Substrate Solution for each well:

Component	Substrate Solution (µL)
Assay Buffer XLII/Tyrosinase Assay Buffer	**23**
Tyrosine/Tyrosinase Substrate	**2**
Tyrosinase Enhancer	**5**

- Add 30 µL of Tyrosine/Tyrosinase Substrate Solution to each of S, EC, IC and SC wells.
- Measure plate in a kinetic mode at OD = 510 nm for 30 - 60 minutes at 25°C.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16.FAQ

17.NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)