ab284539 – Laccase Activity Assay Kit (Colorimetric)

For the sensitive quantification of viable cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284539

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, store the kit components as per the respective temperatures mentioned below. Kit has a storage time of 1 year.

Materials Supplied

Item	Quantity	Storage Condition
Laccase Assay Buffer	25 mL	-20°C
Laccase Positive Control	1 vial	-20°C
Laccase Substrate	10 mL	-20°C

Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Deionized water

Reagent Preparation

Laccase Assay Buffer: Warm to room temperature (RT) before use.

Laccase substrate: Thaw at RT. Divide into aliquots and store at 4°C in amber vials or bottles. Keep at RT, protected from light when in use.

Laccase positive control: Reconstitute in 44 µL Laccase Assay Buffer. Divide into aliquots and store at -20°C. Always keep on ice when in use.

Assay Protocol

Sample preparations:

1. Homogenize plant or fungal tissue (100 mg) with 400 µL Laccase Assay Buffer. Tissues containing cell walls, such as plant leaf, should be grounded mechanically in Laccase Assay Buffer to break down the cell wall.
2. Keep on ice for 10 min followed by centrifugation at 10,000 x g and 4°C for 15 min.
3. Collect the supernatant and estimate the protein concentration using any preferred method. Protein concentration should range between 0.5 - 5 µg/µL.
4. Dilute the lysate, if needed using Laccase Assay Buffer. Use Spin Desalting Columns for removal of small molecules that might interfere with the assay.
5. Prepare two wells for each Sample type labelled as Sample Background Control (SBC), and Sample (S).
6. Add 2-10 µL Samples (up to 10 µg protein) into each of these wells.
7. Adjust the volume to 20 µL/well with Laccase Assay Buffer.
8. For Positive Control, add 4 µL of the provided Laccase Positive Control into the desired well(s) and adjust the volume of to 20 µL/well with Laccase Assay Buffer.
9. For Substrate Control wells, add 20 µL of Laccase Assay Buffer.

Δ Note: *We recommend using the Samples for activity analysis immediately. Otherwise, store the Sample(s) at -80°C for 3-4 days*
Δ Note: *For Unknown Samples, we suggest testing several concentrations of the Sample.*

Reaction Mix:
Add 80 µL Laccase Substrate to the Substrate Control, Sample and Positive Control wells and 80 µL Laccase Assay Buffer to the Sample Background Control wells respectively.

Δ Note: *Have the plate reader ready at OD 420 nm in kinetic mode at 37°C set to record OD at every 30 sec.*

Measurement

Immediately, start recording the absorbance at 30 sec intervals for 20 - 30 min at 37°C.

Calculation:

1. Subtract the SBC reading from Sample readings. If the Substrate Control reading is higher than the SBC reading, subtract the Substrate Control reading from the Sample readings instead.
2. Choose any two time points within the linear portion of the reaction (t_1 & t_2) for each Sample type.
3. Use the Molar Extinction Coefficient for the oxidized product and the Path Length of the reaction well to calculate the molar concentration of the oxidized product formed during the enzymatic reaction for each Sample. Molar Concentration = O.D / (Molar Extinction Coefficient x Path Length). Molar Extinction Coefficient (420 nm) = 36000 M^{-1} cm^{-1}. Path Length for 100 µl reaction = 0.29 cm.
4. Calculate the Amount of Oxidized Product formed (M). M (no. of moles) = Molar Concentration x Reaction Well Volume in Liters (V) = Molar Conc. x 0.0001 No. of pmol = No. of mol x 10^{12}
5. Sample Laccase Activity may be calculated using the following equation:

$$\text{Sample Laccase Activity} = \frac{\Delta M}{(\Delta t \; x \; P)_{(\text{pmol} \,/\, (\text{min x µg}))}} = \text{µUnits / µg or mUnits / mg}$$

Where: **ΔM** = Linear change in oxidized product concentration during Δt (in pmol)
Δ**t** = t2 - t1 (in min)
P = Sample protein content added to well (in µg)

Unit Definition: One unit of Laccase is the amount of enzyme that produces1 µmol of oxidized product per minute at pH 4 at

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)