



Laccase Activity Assay Kit (Colorimetric)

01/20

(Catalog # K2038-100; 100 assays; Store at -20°C)

I. Introduction:

Laccases (EC 1.10.3.2), also known as *p*-benzenediol: oxygen oxidoreductases are a class of copper-containing enzymes found in bacteria, insects, plants and fungi. They catalyze the oxidation of aromatic compounds using molecular oxygen as the terminal electron acceptor and their molecular mass ranges from 60-100 kDa. Laccases play a key role in lignin synthesis and degradation and are utilized in several biotechnological applications such as degradation of dyes, chemical wastes, xenobiotics and other phenolic compounds. Additionally, Laccases are used in food industry for producing cost-effective, healthy foods. Furthermore, they are also being investigated in the construction of Laccase biosensors because of their high redox potential. **BioVision's Laccase Activity Assay Kit** is a simple, one step, plate based assay to measure Laccase activity in various samples. In this assay, samples containing Laccase will oxidize the substrate to generate a colored product with a strong absorbance at 420 nm. The kit is rapid, easy to use and high-throughput adaptable. It can measure Laccase activity as low as 20 µU in samples.



II. Application:

Measurement of Laccase activity using a 96-well plate format

III. Sample Types:

- Fungal lysate
- · Plant lysate
- Recombinant enzyme
- Purified protein

IV. Kit Contents:

Components	K2038-100	Cap Code	Part Number
Laccase Assay Buffer	25 ml	NM	K2038-100-1
Laccase Substrate	10 ml	Amber/NM	K2038-100-2
Laccase Positive Control	1 vial	Blue	K2038-100-3

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay. All kit components are stable for at least twelve months.

- 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.

VII. Laccase Activity Assay Protocol:

1. Sample preparation: 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. Keep on ice for 10 min followed by centrifugation at 10,000 x g and 4°C for 15 min. Collect the supernatant and estimate the protein concentration using any preferred method. We recommend using BCA Protein Assay Kit (BioVision Cat # K813-2500). Protein concentration should range between 0.5 - 5 µg/µl. Dilute the lysate, if needed using Laccase Assay Buffer. Use EZ-Desalt™ Spin Desalting Columns (BioVision Cat # 6564-25) for removal of small molecules that might interfere with the assay. Prepare two wells for each Sample type labeled as Sample Background Control (SBC), and Sample (S). Add 2-10 µl Samples (up to 10 µg protein) into each of these wells. Adjust the volume to 20 µl/well with Laccase Assay Buffer. 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. For Substrate Control wells, add 20 µl of Laccase Assay Buffer.

Notes:

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

2. 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.

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

- 3. Measurement: Immediately, start recording the absorbance at 30 sec intervals for 20 30 min at 37°C.
- 4. Calculation: 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. Choose any two time points within the linear portion of the reaction (t₁)





& t₂) for each Sample type. Use the **Molar Extinction Coefficient** for the oxidized product and the **Path Length** of the reaction well (given below) to calculate the **molar concentration of the oxidized product** formed during the enzymatic reaction for each Sample.

Molar Extinction Coefficient (420 nm) = $36000 \text{ M}^{-1}\text{cm}^{-1}$ Path Length for 100 µl reaction = 0.29 cm

Molar Concentration = O.D / (Molar Extinction Coefficient x Path Length)

Calculate the Amount of Oxidized Product formed (M) as shown below:

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}

Calculate ΔM , which is the change in amount of oxidized product formed between time t₁ and t₂.

Sample Laccase Activity may be calculated using the following equation:

Sample Laccase Activity = $\Delta M / (\Delta t \times P) (pmol / (min \times \mu g)) = \mu Units / \mu g or mUnits / mg$

Where: ΔM = Linear change in oxidized product concentration during Δt (in pmol)

 $\Delta \mathbf{t} = \mathbf{t}_2 - \mathbf{t}_1 \text{ (in min)}$

P = Sample protein content added to well (in μ g)

Unit Definition: One unit of Laccase is the amount of enzyme that produces1 µmol of oxidized product per minute at pH 4 at 37°C.



Figures: (a). Enzyme kinetics using mushroom lysate (5 µg protein per well) and potato lysate (0.62 µg protein per well) (b). Laccase specific activity in potato and mushroom lysate. Experiments were conducted according to kit protocol.

VIII. Related Products:

Hydrogen Peroxide Colorimetric/Fluorometric Assay Kit (K265) Human Lactate Dehydrogenase A Inhibitor Screening Kit (Colorimetric) (K492) Lactate Dehydrogenase Activity Colorimetric Assay Kit (K726) Tyrosinase Activity Assay Kit (Colorimetric) (K742) Protein Disulfide Isomerases (PDI) Inhibitor Screening Kit (Fluorometric) (K840) Protein Disulfide Isomerase (PDI) Activity Assay Kit (Fluorometric) (K949)

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