Lysozyme Detection Kit
(Turbidimetric method)

Ordering info

TBK0528, 100 assays (using cuvettes)

Description

Lysozyme, also known as muramidase or N-acetylmuramic hydrolase, is a small, monomeric enzyme that hydrolyzes the β-1,4-linkages between N-acetyl-d-glucosamine and N-acetylmuramic acid residues in the peptidoglycan of bacterial cell walls. The cell lysis, or bursting, that usually follows, is the basis for its bactericidal activity in vivo.

The assay method typically employed to measure lysozyme activity is based on the lysis of *Micrococcus luteus* cells following the hydrolysis of the substrate, the protective cell wall of this organism, by lysozyme.

The turbidimetric methods are among the simplest and quickest to perform and rely on the spectrophotometric measurement of the suspensions of *M. luteus*. During the incubation of the lysozyme sample and substrate (*M. luteus*), the reaction is followed by monitoring the decrease in absorbance at 450 nm.

The Lysozyme Detection Kit provides ready-to-use reagents for detecting the presence of lysozyme activity. This simple assay detects lysozyme activity using *Micrococcus luteus* cells as the substrate.

Features

- Simple: It allows for the quick assessment of lysozyme activity without the need for complex or time-consuming preparation steps.
- Spectrophotometric Analysis: The change in turbidity is monitored spectrophotometrically, usually at an absorbance of 450 nm. This provides an objective and quantifiable measure of lysozyme activity.
- Sensitivity: Turbidimetric assays are sensitive and can detect variations in lysozyme activity effectively.
- Low Equipment Requirements: This method requires basic laboratory equipment like a spectrophotometer and a cuvette, making it accessible to most laboratories.
- Cost-Effectiveness.

Kit Components

Components	TBK0528
LYS Assay Buffer	2 x125 mL
Lysozyme Control [a]	2 g
Micrococcus luteus cells	5 tubes

Order Info Kit Components: *LYS Assay Buffer (TBK0528-B) | Lysozyme Control (TBZ0312-2g) | Micrococcus luteus cells (TBC0023).*

[a] Lysozyme from chicken egg white, 20,000 units/mg.

One unit is defined as the amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25°C, using a suspension of *Micrococcus luteus* as the substrate.

Storage

All the reagents should be kept according to the labels on vials. The *Micrococcus luteus* cells (pellet) should be stored at -20°C upon receipt while the other components should be at 4°C.

Quality Control

The kit is tested in a functional assay with Lysozyme enzyme dilutions.

Applications

Lysozyme Activity Kit has been used to measure the catalytic activity of lysozyme enzyme.

Material required (not included)

- Cuvettes or 96 well plates
- Pipettes and tips
- Appropriate instrument to measure absorbance at 450 nm (A_{450}) at constant temperature of 25 °C.

Technical Assistance

Please refer any technical questions to support@tiarisbiosciences.com

PROTOCOL I

I. REAGENT PREPARATION

LYS Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use.

Micrococcus luteus* cell suspension:** Prepare an initial cell suspension of ***Micrococcus luteus.
Add 1 ml of **LYS Assay Buffer** to a vial containing the cell pellet and homogenize with pipettes.
***M. luteus* Working Suspension:** It is recommended the final cell suspension used in the assay has an A450 between 0.6–0.7 versus an **Assay Buffer** blank.
*Dilute the initial suspension 1:25 in **LYS Assay Buffer** to obtain a suspension with an OD at 450 nm between 0.6 – 0.7. (Add 1 ml in 24 ml of **LYS Assay Buffer**)*
Lysozyme Control Stock: Prepare a 50mg/ml stock solution of Lysozyme in **LYS Assay Buffer.** Aliquot Lysozyme control so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months.
Working Lysozyme Control solution: Immediately before use, prepare a solution containing 400-500 units/mL of Lysozyme in cold **LYS Assay Buffer.**

II. PROCEDURE (using cuvettes)

The researcher must determine the optimal procedure conditions for the lysozyme specific to their application.

The following protocol describes the assay of lysozyme activity in a total volume of 830 µL per cuvettes. The kit provides sufficient material for 100 assays.

1. Pipette 800 µL of the ***M. luteus* Working Suspension** into one cuvette for a blank, one for a control, and one for each sample.
2. Equilibrate the cuvettes to 25 °C.
3. Monitor the A_{450} until constant using a suitably thermostatted spectrophotometer.
4. Add 30 µL of **LYS Assay Buffer** to the blank cuvette, 30 µL of Lysozyme Control to the control cuvette, and 30 µL of Test Sample to the remaining cuvettes.
5. Immediately mix by inversion and record the decrease in A_{450} for ~ 5-10 minutes. Obtain the maximum linear rate (ΔA_{450}/minute) for both the test and the blank.

III. RESULTS

Calculate units/mL enzyme:

$$\frac{(\Delta A_{450}/\text{min Test} - \Delta A_{450}/\text{min Blank})\,(df)}{(0.01)\quad(0.03)}$$

df = dilution factor

0.001 = ΔA_{450} as per the Unit Definition

0.03 = Volume (in milliliters) of enzyme solution

$$\text{units/mg solid} = \frac{\text{units/mL enzyme}}{\text{mg solid/mL enzyme}}$$

PROTOCOL II

I. KINETIC 96- WELL TURBIDIMETRIC LYSOZYME ASSAY (96 well plates).

PROCEDURE:

1. Prepare Standards: Dilutions of lysozyme should be prepared fresh daily from frozen aliquots using LYS Assay Buffer. Prepare a two-fold dilution series.
   Add lysozyme diluted to a final volume of 25 µL to wells of a flat-bottomed 96-well plate, in triplicate.
2. Test samples (25 µL) should be placed into wells of a 96-well plate.
3. 175 µL of the working solution of *Micrococcus luteus* suspension prepared in LYS Assay Buffer will quickly be added to each well. Immediately place the plate into the plate reader and rapid mixing, the change in turbidity will be measured every 30 seconds for 5 minutes at 450 nm.
4. Following data collection, calculate the average OD of triplicate wells at each timepoint for the set of technical replicates.
5. Obtain the slope using the average OD at each timepoint vs. time (minutes) for the consistently linear portion of the experiment.
6. Convert slopes to units, where 1 unit is equivalent to a decrease in absorbance of 0.001/min^{-1}.
7. Subtract the units for a given lysozyme from the units for the corresponding buffer control and adjust according to the amount of enzyme present in the reaction, resulting in a measure of activity in Units μg^{-1}.
8. To improve the reliability of the activity values, perform the experiment two additional times using independent substrate preparations. Calculate final activity values and standard deviations for the average of each experiment for each experimental condition.