3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Lysozyme Detection Kit

Catalog Number **LY0100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

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

Lysozyme activity results in the lysis of the *Micrococcus lysodeikticus* cells. During incubation of the lysozyme sample and substrate, the reaction is followed by monitoring the decrease in absorbance at 450 nm.

The assay can be performed using cuvettes or plates. The procedure provided here is for detection using cuvettes with a 0.83 mL reaction volume. One publication has used this kit with 96 well plates.[2]

Components

Each kit contains sufficient reagents for 100 assays using cuvettes (0.83 mL assay volume).

Reaction Buffer (Catalog Number L9295) 2 × 500 mL
66 mM potassium phosphate,
pH 6.24 at 25 °C
Provided as a ready-to-use solution
(no further dilution required)

Micrococcus lysodeikticus cell suspension 5 g
(Catalog Number M3770)

Lysozyme Control 2 × 1 g
(Catalog Number L6876)

Reagents and Equipment Required but Not Provided

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Micrococcus lysodeikticus cell suspension: Prepare an initial cell suspension of *Micrococcus lysodeikticus* (Catalog Number M3770) in Reaction Buffer (Catalog Number L9295), using scaled amounts of the *Micrococcus lysodeikticus* and the Reaction Buffer as appropriate for the particular procedure. For example, 10 mL is one suggested volume for preparing cell suspensions.

It is recommended the final cell suspension used in the assay has an A_{450} between 0.6–0.7 versus a Reaction Buffer blank. Historically, this final suspension can be prepared at 0.01 % (w/v). For example, to prepare 10 mL of a 0.01% (w/v) suspension, 1 mg of cells would be used per 10 mL of Reaction Buffer. However, this % value can be different, depending on the batch of the parent cell suspension used.

Initial suspensions of the *Micrococcus lysodeikticus* in the Reaction Buffer can be prepared at higher initial suspension concentrations (e.g. 0.1%). Titers can then be prepared to obtain a suitable suspension in the desired A_{450} range.

Lysozyme Solution (enzyme control): Immediately before use, prepare a solution containing 200–400 units/mL of Lysozyme (Catalog Number L6876) in cold Reaction Buffer (Catalog Number L9295). Mix briefly to ensure the lysozyme is dissolved. Consult the Certificate of Analysis (CofA) for the specific activity value of the specific batch of lysozyme control. The enzyme is supplied at a minimum concentration of 40,000 units/mg, so serial dilutions are recommended.

Test Samples: Immediately before use, prepare a solution containing 200–400 units/mL of Lysozyme in cold Reaction Buffer (Catalog Number L9295). Mix briefly to ensure the lysozyme is dissolved.

Storage/Stability
The Reaction Buffer is stable for at least 2 years at 2–8 °C.

The Lysozyme (Catalog Number L6876) may be stored long term at –20 °C and remains active for 4 years.

The *Micrococcus lysodeikticus* cells (Catalog Number M3770) should be stored long term at –20 °C.

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

1. Pipette 800 µL of the *Micrococcus* cell 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 Reaction Buffer to the blank cuvette, 30 µL of Lysozyme Solution 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 minutes. Obtain the maximum linear rate (ΔA_{450}/minute) for both the test and the blank.

Results
Calculate units/mL enzyme:

$$\frac{(\Delta A_{450}/\text{min Test} - \Delta A_{450}/\text{min Blank})\ (df)}{(0.001)\ (0.03)}$$

df = dilution factor
0.001 = ΔA_{450} as per the Unit Definition
0.03 = Volume (in milliliters) of enzyme solution

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

Unit Definition: Sigma's typical unit definition for lysozyme is based on an assay with a total volume of 2.6 mL performed in a cuvette format. One unit will produce a ΔA_{450} of 0.001 per minute at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate, in a 2.6 mL reaction mixture (1 cm pathlength).

Unlike most enzymatic unit definitions which are based on moles of substrate converted or product produced, this unit definition is dependent on the reaction volume. For comparison of activity from different samples, the reaction volume and pathlength must be constant.

Reference
1. Shugar, D., *Biochim. Biophys. Acta*, **8**, 302-309 (1952).
2. Wang, T. *et al.*, *Chem. Sci.*, **7**, 3234-3239 (2016).

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