Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Lysozyme Activity Assay Kit (Fluorometric)

rev 04/21

(Catalog # K236-100; 100 assays; Store at -20 °C)

I. Introduction:

Lysozyme (EC 3.2.1.17), also known as muramidase or *N*-acetylmuramide glycanhydrolase is a hydrolase acting on glycosidic bonds. It hydrolyzes the β-(1-4)-glucosidic linkage between *N*-acetyl-muraminic acid and *N*-acetyl-D-glucosamine residues present in the mucopolysaccharide cell wall. Lysozyme is ubiquitously found in a wide range of biological fluids such as tears, saliva and tissues. It exhibits antibacterial, antitumor and immune modulatory activities. Elevated concentrations of lysozyme in urine and serum have been reported in patients suffering leukemia, tuberculosis, megaloblastic anemias, acute bacterial infections, ulcerative colitis, severe renal insufficiency, pyelonephritis and nephritis. **BioVision's Lysozyme Activity Assay Kit** utilizes the ability of lysozyme to cleave a synthetic substrate to release the free fluorophore, which can be easily quantified (Ex/Em= 360/445 nm). This kit provides a simple, ultra-sensitive assay that can detect as low as 2 µU/ml of Lysozyme activity in a variety of biological samples.

$$\text{Lysozyme Substrate} \xrightarrow{\text{Lysozyme}} \text{Cleaved Substrate + Fluorescent Product (Ex/Em= 360/445 nm)}$$

II. Application:

- Measurement of lysozyme activity in various biological samples/preparations

III. Sample Types:

- Cells: e.g. HepG2, J774
- Tissues: e.g. Spleen, Kidney
- Biological fluids: e.g. Serum, Tears, Saliva
- Bacteria and Yeast

IV. Kit Contents:

Components	K236-100	Cap Code	Part Number
Lysozyme Assay Buffer	25 ml	WM	K236-100-1
Lysozyme Stop Buffer	25 ml	NM	K236-100-2
Lysozyme Substrate (in DMSO)	65 µl	Red	K236-100-3
Lysozyme Positive Control (lyophilized)	1 vial	Green	K236-100-4
4-Methylumbelliferone Standard (5 mM)	35 µl	Yellow	K236-100-5

V. User Supplied Reagents and Equipment:

- 96-well white opaque plate
- Multi-well spectrophotometer (fluorescence plate reader)
- Protease inhibitor cocktail (BioVision Cat. # K272 or equivalent)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Lysozyme Assay Buffer:** Bring to room temperature (RT) before use. Store at either 4 °C or -20 °C.
- **Lysozyme Stop Buffer:** Bring to RT before use. Store at either 4 °C or -20 °C.
- **Lysozyme Substrate**: Aliquot and store at -20 °C.
- **Lysozyme Positive Control:** Reconstitute with 110 µl Lysozyme Assay buffer, pipet up and down to mix thoroughly. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Stable for 2 months after reconstitution.
- **4-Methylumbelliferone Standard:** Light sensitive. Store at -20 °C, protected from light. Use within two months.

VII. Lysozyme Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize cells (~1 X 10^7) or tissue (~10-50 mg) with 100 µl of ice cold Lysozyme Assay Buffer containing protease inhibitor cocktail (BioVision Cat. # K272 or equivalent) and keep on ice for 10 min. Centrifuge Samples at 12,000 x g at 4 °C for 5 min and collect the supernatant. Add 2-40 µl of Sample into desired well(s) in a white 96-well plate as Sample; add same volume of Lysozyme Assay Buffer into separate well as Reagent Background Control. For Positive Control, add 8-10 µl of Lysozyme Positive Control into desired well(s). Adjust the volume of Positive Control, Reagent Background Control and Sample wells to 40 µl/well with Lysozyme Assay Buffer.

   Notes:

   a. For Unknown Samples, we recommend doing pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.

   b. For fungi, pulmonary or gastrointestinal samples, it is strongly recommended to treat Samples with a lysozyme competitive inhibitor (i.e. N,N'N''-triacetylchitotriose, 10 mM, not provided).

2. **Standard Curve Preparation:** Prepare a 100 µM 4-Methylumbelliferone (4-MU) by adding 10 µl of 5 mM 4-MU to 490 µl Lysozyme Assay Buffer. Further dilute the 100 µM Standard solution by adding 10 µl of 100 µM to 90 µl Lysozyme Assay Buffer to generate 10 µM 4-MU Standard. Add 0, 2, 4, 6, 8, 10 µl of 10 µM 4-MU standard into a series of wells to generate 0, 20, 40, 60, 80, 100 pmol of 4-MU/well respectively. Adjust the volume to 50 µl/well with Lysozyme Assay Buffer.

Note:

a. The 100 µM 4-MU is stable for 2 months at -20 °C, protected from light.

3. **Substrate Hydrolysis:** Mix 4 µl of the Lysozyme Substrate with 60 µl of Lysozyme Assay Buffer, vortex briefly and keep at RT. Add 10 µl of prepared Substrate to each well containing the Test Samples, Reagent Background Control and Lysozyme Positive Control. Mix well. Incubate the plate at 37 °C for 30-60 min, protected from light. After incubation time, add 50 µl Lysozyme Stop Buffer to each well containing Samples, Positive Control, Background Control and Standards. Mix well.

Notes:

a. For Samples exhibiting significant background, prepare a parallel Samples well(s) as Sample Background Control. Add 10 µl of Lysozyme Assay Buffer to the Sample Background Control well.

b. Incubation time depends on the Lysozyme enzymatic activity in Samples. Longer incubation time may be required for Samples having low Lysozyme activity.

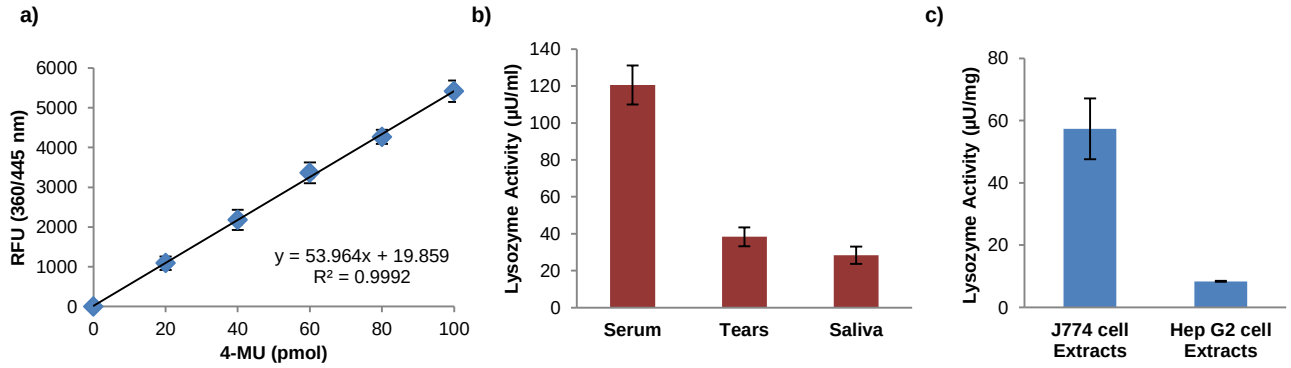
4. **Measurement:** Measure the fluorescence intensity at Ex/Em= 360/445 nm at 37 °C in end point setting using a fluorescence microtiter plate reader.
5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the 4-MU Standard Curve. Subtract the Reagent Background Control from Sample reading(s). If the Sample Background Control reading is higher than the Reagent Background Control, subtract Sample Background Control instead. Apply Sample ΔRFU to 4-MU Standard Curve to obtain the corresponding pmol of product formed (**B**, in pmol). Calculate the activity of Lysozyme in the Sample as:

$$\textbf{Sample Lysozyme Activity} = B/(\Delta T \times V) \times D = \text{pmol/min/ml} = \mu\text{U/ml}$$

Where: **B** = 4-MU from the Standard Curve (pmol)
Δ**T** = Reaction time (min.)
V = Sample volume added into the reaction well (ml)
D = Dilution Factor

Note: For samples using lysozyme inhibitor (see VII. 1. Notes: b), subtract the Sample with inhibitor reading from Sample readings. Apply the Sample ΔRFU to 4-MU Standard Curve to obtain the corresponding pmol of product formed (**B**, in pmol), and calculate the activity of Lysozyme in the samples using the above mentioned formula. Lysozyme specific activity can be expressed as U/mg of protein

Unit Definition: One unit of lysozyme activity is the amount of enzyme that generates 1.0 µmol of 4-MU per min., at pH 5.0 at 37 °C.



Figures: (a) 4-Methylumbelliferon Standard Curve, results from three independent experiments. (b) and (c) Measurement of Lysozyme activity in human samples and cell cultured samples. Undiluted pooled serum (10 µl), pooled tears (15 µl), pooled saliva (30 µl) or J774 cells (15 µg), HepG2 cells (80 µg) were incubated with Lysozyme Substrate for 60 min. All assays were performed following kit protocols.

VIII. RELATED PRODUCTS:

Lysozyme Inhibitor Screening Kit (K220)
Lysozyme (M1237)
OryzaExp™ Lysozyme, Human Recombinant (P1400)
EZlys™ Lysozyme, Human (8005)
Lysozyme Antibody (A1110)
Lysozyme ELISA Kit (K4243)

FOR RESEARCH USE ONLY! Not to be used on humans.