FOR RESEARCH USE ONLY!

Lysozyme Activity Assay Kit (Fluorometric)

(Catalog #BN00504; 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. Assay Genie'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. Applications:

- Measurement of lysozyme activity in various biological samples/preparations

III. Sample Type:

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

IV. Kit Contents:

Components	BN00504-100	Cap Code	Part Number
Lysozyme Assay Buffer	25 ml	WM	BN00504-1
Lysozyme Stop Buffer	25 ml	NM	BN00504-2
Lysozyme Substrate (in DMSO)	65 µl	Red	BN00504-3
Lysozyme Positive Control (lyophilized)	1 vial	Green	BN00504-4
4-Methylumbelliferone Standard (5 mM)	35 µl	Yellow	BN00504-5

V. User Supplied Reagents and Equipment:

- 96-well white opaque plate
- Multi-well spectrophotometer (fluorescence plate reader)
- Protease inhibitor cocktail

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. Aliquot 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. 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 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. For Positive Control, add 8-10 µl of Lysozyme Positive Control into desired well(s). Adjust the volume of Positive Control, 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).

   c. For samples exhibiting significant background, prepare parallel samples well(s) as background controls.

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.

Notes:

The 100 µM 4-MU is stable for 2 months at -20°C, protect 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 and Lysozyme positive control. Mix well. Incubate 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.

   Note:

   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 fluorescence intensity at Ex/Em= 360/445nm at 37°C with 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; apply sample ΔRFU to 4-MU Standard Curve to obtain the corresponding pmol of product formed (**B**, in pmol) and calculate the activity of Lysozyme activity in the sample as:

$$\textbf{Sample Lysozyme} = B/(\Delta T \times V) \times D = \text{nmol/min/ml} = \text{mU/ml}$$

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

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.

a)

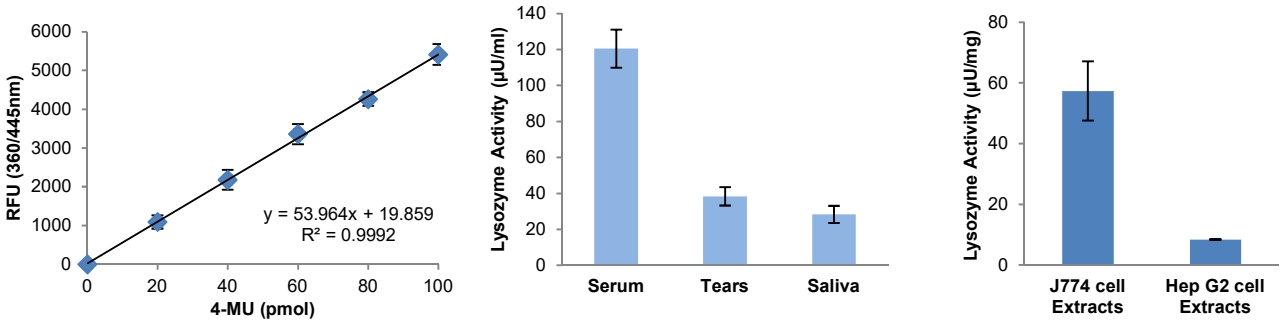

Figure: (a) 4-Methylumbelliferon Standard Curve, results show results from three independent experiments. (b) and (c) Measurement of Lysozyme activity in human samples and cell cultured samples. Undiluted pooled serum (10 µl), pooled tears (15 µl), pooled saliva (30 µl) or J774 cells (15 µg), HepG2 cells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

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