Lysozyme Activity Assay Kit

ARG83554 Lysozyme Activity Assay Kit can be used to measure Lysozyme in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids

Catalog number: ARG83554

Package: 100 tests

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PRINCIPLE OF THE ASSAY

ARG83554 Lysozyme Activity Assay 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the Standard, Substrate at-20 °C, other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	-20 °C
Assay Buffer	4X 30 ml	4 °C
Reaction Buffer	20 ml	4 °C
Substrate	1 vial (lyophilized)	-20 °C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the Standard, Substrate at-20 °C, other component at 2-8°C. Use the kit before expiration date.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and Bacteria-Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate with 1 ml Assay buffer per 5×10^6 cell or bacteria. Centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Standard:** Add **0.5 ml** of Assay Buffer to yield stock **20000 U/ml** standard.

- **Substrate:** Reconstitute the Substrate with **3 ml** of Reaction Buffer. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **160 µl** Reaction Buffer into each wells.
2. Add **30 µl** Substrate into each wells.
3. Add **10 µl** Sample, Assay Buffer and Standard into respective wells.
4. Mix well. Incubate at **RT** for **5 min**. Read the OD at **450nm**.

Summary of Lysozyme Activity Assay Kit Procedure

Reagent	Sample	Standard	Blank
Reaction Buffer	160 µ	160 µ	160 µ
Substrate	30 µ	30 µ	30 µ
Sample	10 µl		-
Assay Buffer	-	10 µl	-
Standard		-	10 µl
Mix well. Incubate at **RT** for **5 min**. Read the OD at **450nm**			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.
2. Calculation:

A. Definition:

$C_{Standard}$: the concentration of standard, 20000 U/ml

$C_{Protein}$: the protein concentration, mg/ml

W: the weight of sample, g

V_{Sample}: the volume of sample, 0.01 ml

$V_{Standard}$: the volume of standard, 0.01 ml

V_{Assay}: the volume of Assay buffer, 1 ml

N: the quantity of cell or bacteria, $N \times 10^4$

B. Formula:

a). According to the protein concentration:

Lysozyme (U/mg) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / [(OD_{Blank} - OD_{Standard}) \times V_{Sample} \times C_{Protein}]$

$=20000 \times (OD_{Blank} - OD_{Sample}) / [(OD_{Blank} - OD_{Standard}) \times C_{Protein}]$

b). According to the weight:

Lysozyme Activity (U/g) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / [(OD_{Blank} - OD_{Standard}) \times (V_{Sample} \times W / V_{Assay})]$

$=20000 \times (OD_{Blank} - OD_{Sample}) / [(OD_{Blank} - OD_{Standard}) \times W]$

C). According to the quantity of cells or bacteria:

Lysozyme Activity ($U/10^4$) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Blank} - OD_{Sample}) / [(OD_{Blank} - OD_{Standard}) \times (V_{Sample} \times N / V_{Assay})]$

$=20000 \times (OD_{Blank} - OD_{Sample}) / [(OD_{Blank} - OD_{Standard}) \times N]$