Version 3a, Last updated 11 May 2023

ab211113 Lysozyme Activity Assay Kit

For the rapid, sensitive and accurate measurement of lysozyme activity in a variety of biological samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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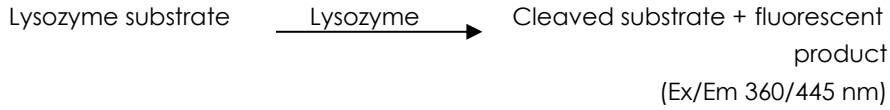
1. Overview

Lysozyme Activity Assay Kit (ab211113) provides a convenient method for quantifying Lysozyme activity in mammalian cell or tissue lysates, biological fluids such as tears or saliva. This product can also be used to measure lysozyme activity in yeast and bacteria.

The assay is based on the ability of lysozyme to cleave a synthetic substrate and release a free fluorophore which can be easily quantified at Ex/Em= 360/445 nm in a fluorescent microplate reader.

This kit provides a simple, ultra-sensitive assay that can detect as low as 2 µU/mL of Lysozyme activity.



Lysozyme (Muramidase, N-acetylmuramide glycanhydrolase; EC 3.2.1.17) catalyzes the hydrolysis of the β-(1-4)-glycosidic linkage between N-acetyl-muraminic acid and N-acetyl-D-glucosamine residues present in the bacterial mucopolysaccharide cell wall. Lysozyme is ubiquitously found in a wide range of biological fluids such as tears, saliva and mucus. It is also present in cytoplasmic granules of macrophages and PMN cells. 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.

2. Protocol Summary

Standard curve preparation

Sample preparation

Substrate hydrolysis step

Stop reaction

Measure fluorescence (Ex/Em = 360/445 nm) at 37°C
In end point mode

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer XXI/Lysozyme Assay Buffer	25 mL	-20°C	4°C / -20°C
Lysozyme Stop Buffer	25 mL	-20°C	4°C / -20°C
Lysozyme Substrate/Lysozyme Substrate (in DMSO)	65 µL	-20°C	-20°C
Lysozyme Positive Control	1 vial	-20°C	-20°C
4-Methylumbelliferone Standard/4-Methylumbelliferone Standard (5 mM)	35 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 360/445 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate opaque white
- Dounce homogenizer (if using tissue)
- Protease Inhibitor Cocktail (ab65621)
- (Optional) BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer XXI/Lysozyme Assay Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 Lysozyme Stop Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.3 Lysozyme Substrate/Lysozyme Substrate (in DMSO) (65 µL):

Ready to use as supplied. Warm by placing in a 37°C bath for 1-5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the probe is thawed, use within two months.

9.4 Lysozyme Positive Control (lyophilized):

Reconstitute in 110 µL Assay Buffer XXI/Lysozyme Assay buffer. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 4-Methylumbelliferone Standard/4-Methylumbelliferone Standard (5 mM) (35 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Reagent is light sensitive and it needs to be protected from light (wrap vial in foil). Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Use within two months.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 100 µM 4-Methylumbelliferone (4-MU) working standard solution by adding 10 µL of 4-MU/5 mM 4-MU to 490 µL Assay Buffer XXI/Lysozyme Assay Buffer.

10.2 Prepare a 10 µM 4-MU standard by diluting 10 µl of the 100 µM Standard solution in 90 µL Assay Buffer XXI/Lysozyme Assay Buffer.

10.3 Using 10 µM 4-MU Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	4-MU 10 µM standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount 4-MU in well (pmol/well)
1	0	150	50	0
2	6	144	50	20
3	12	138	50	40
4	18	132	50	60
5	24	126	50	80
6	30	120	50	100

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Add protease inhibitor to sample buffer immediately prior to use.
- If using fungi, pulmonary or gastrointestinal samples, we strongly recommend treating samples with a lysozyme competitive inhibitor (ie. N,N'N''-triacetylchitotriose at 10 mM).

11.1 Cell lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation: 1 x 10^7 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 100 µL ice cold Assay Buffer XXI/Lysozyme Assay buffer (containing protease inhibitors).
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Keep on ice for 10 minutes.
11.1.6 Centrifuge sample for 5 minutes at 4°C at 12,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new tube.
11.1.8 Keep on ice.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation ~10 - 50 mg).
11.2.2 Wash tissue with cold PBS.
11.2.3 Homogenize tissue in 100 µL of ice cold Assay Buffer XXI/Lysozyme Assay Buffer (containing protease inhibitors) with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.4 Centrifuge sample for 5 minutes at 4°C at 12,000 *xg* using a cold microcentrifuge to remove any insoluble material.
11.2.5 Collect supernatant and transfer to a new tube.
11.2.6 Keep on ice.

11.3 Biological fluids (serum, tears, saliva):

Biological fluids can be measured directly. However, we recommend a quick centrifugation step for 5 minutes at 4°C at 12,000 *xg* to discard any debris found in the sample.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: If you suspect your samples contain substances that can generate background, set up Sample Background Controls to correct for background noise.

12.1 Plate Loading:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2-40 µL samples (adjust volume to 40 µL/well with Assay Buffer).
- Sample Background Control wells = 2-40 µL Sample(s) (adjust volume to 40 µL/well with Assay Buffer).
- Reagent Background Control = 2-40 µL Assay Buffer XXI/Lysozyme Assay Buffer (adjust volume to 40 µL/well with Assay Buffer).
- Positive control = 8-10 µL Lysozyme Positive Control (adjust volume to 40 µL with Assay Buffer).

12.2 Substrate Hydrolysis:

12.2.1 Mix 4 µL Lysozyme Substrate with 60 µL Assay Buffer. Vortex briefly and keep at RT.

12.2.2 Add 10 µL of prepared substrate to each well containing samples and Lysosome Positive Control. Do not add to Standard or Sample Background Control wells.

Δ Note: Increase amount of prepared substrate if there is not enough for all your tests.

Δ Note: For Samples exhibiting significant background, add 10 µL of Assay Buffer XXI/Lysozyme Assay Buffer to well containing Sample Background Control.

12.2.3 Mix well.

12.2.4 Incubate the plate at 37°C for 30-60 minutes protected from light.

Δ Note: Incubation time depends on the Lysozyme enzymatic activity in samples. Longer incubation time may be required for samples having low Lysozyme activity.

12.2.5 After incubation time, add 50 µL Lysozyme Stop Buffer to all wells. Mix well.

12.3 Measurement:

12.3.1 Measuring immediately fluorescence on a fluorescent microplate reader at Ex/Em=360/445nm at 37°C in end point mode.

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Subtract the mean fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected fluorescence.

13.2 Standard curve calculation:

13.2.1 Average the duplicate reading for each standard.

13.2.2 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.3 Measurement of Lysozyme activity in the sample:

13.3.1 Subtract the sample background control from sample reading if significant.

13.3.2 Subtract the Reagent Background Control from Sample reading(s). If Sample Background Control is higher than Reagent Background Control, subtract Sample Background Control instead.

13.3.3 Apply variation of fluorescence in the sample (ΔRFU) to the Standard curve to get B pmol of 4-MU generated during the reaction.

13.3.4 Lysozyme activity (pmol/min/mL or mU/mL) in the test samples is calculated as:

$$Lysozyme\ \ Activity = \left(\frac{B}{\Delta T * V}\right) * D$$

Where:

B = amount of 4-MU amount in sample well calculated from Standard Curve (pmol).

ΔT = Reaction time (min).

V = Sample volume added into the reaction well (mL)

D = sample dilution factor.

Lysozyme specific activity can be expressed as U/mg of protein.

Unit definition:

1 Unit of Lysozyme activity = amount of enzyme that generates 1.0 µmol of 4-MU per minute at pH5.0 at 37°C

Δ Note: For samples using lysozyme inhibitor (see section on General sample information), 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.

14. Typical Data

Typical standard curve – data provided for **demonstration purposes** only. A new standard curve must be generated for each assay performed.

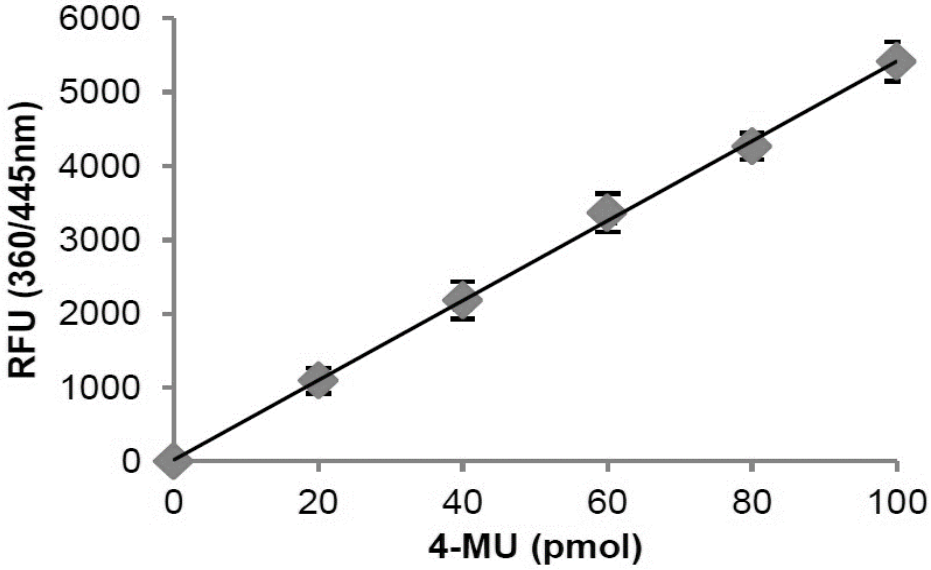


Figure 1. Typical 4-Methylumbelliferon (4-MU) Standard Curve.

Figure 2. Measurement of Lysozyme activity in human fluids. Assays were performed following kit protocols.

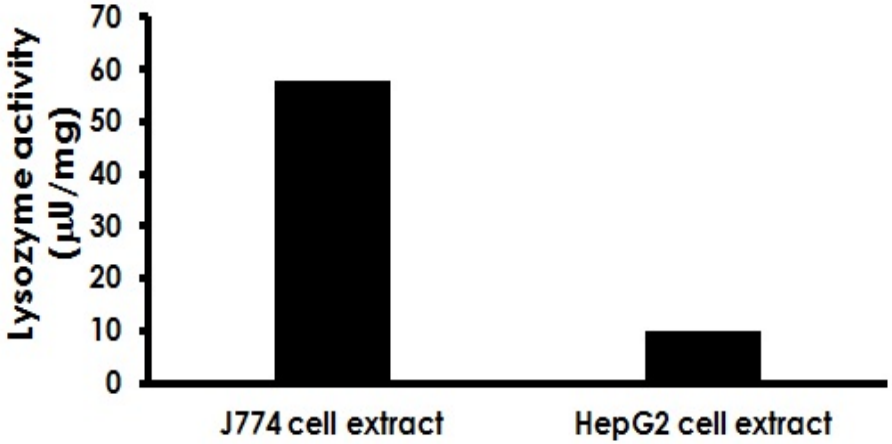


Figure 3. Measurement of Lysozyme activity in human cultured cell lysates. Assays were performed following kit protocols.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare standard dilution [20 – 100 pmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), sample background control samples (40 µL), reagent background control samples (40 µL) sample (40 µL) and positive control wells (40 µL).
- Mix 4 µL Lysozyme Substrate + 60 µL Assay Buffer.
- Add 10 µL Substrate mix to sample and positive control wells.
- Incubate for 30-60 minute at 37°C away from light.
- Add 50 µL Stop Buffer to all wells (standard, sample, sample background control, positive control).
- Measure fluorescence immediately at Ex/Em= 360/445 nm in end point mode.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ dilute sample so it is within the linear range

17. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)