

## ab285338– Lysozyme ELISA Kit

For the quantitative measurement of human Lysozyme.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab285338>

### Storage and Stability

On receipt entire kit should be stored at 4°C, protected from light. Upon opening, use kit within 6 months. Avoid freeze-thaw cycles.

### Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 wells	4°C
Lysozyme Standards (S1 – S7)	7 X 250 µL	-20°C
Lysozyme Controls (C1, C2)	2 X 250 µL	4°C
Anti-Lysozyme Enzyme Concentrate	12 mL	4°C
Sample Diluent	2 X 20 mL	4°C
TMB Substrate	12 mL	4°C
20X Wash Concentrate	25 mL	4°C
Stop Solution	12 mL	4°C

### Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm
- 37 °C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

### Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes

**20X Wash Buffer Concentrate:** Prepare 1X Wash buffer by adding the contents of the bottle (25 mL, 20X) to 475 mL of distilled or deionized water. Store at room temperature (20-25°C)

**Lysozyme Standards:** All standards are ready to use. Check standard value on each vial. This value might vary from lot to lot.

**Lysozyme Controls:** All controls are ready to use. Check control value on each vial.

### Sample Preparation

Collect blood specimens and separate the serum immediately. Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens. Dilute serum samples 1:250 in sample diluent. Dilute stool samples 1:100 in sample diluent.

### Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.
1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal, and store at 2-8°C.
  2. Pipette 25µL of the standards, controls and diluted samples into the assigned well.
  3. Add 100µL of anti-lysozyme enzyme conjugate solution into all wells.
  4. Incubate the plate for 60 minutes at room temperature, with shaking.
  5. Remove liquid from all wells. Wash wells three times with 300 of 1X wash buffer. Blot on absorbent paper towels.
  6. Add 100 µL of TMB substrate solution to all wells
  7. Incubate the plate for 15 minutes at room temperature.
  8. Add 50 µL of Stop solution to each well and gently mix for 15-20 seconds.
  9. Read the absorbance on ELISA Reader of each well at 450nm within 15 minutes after adding the stop solution.

### Calculation:

Check Lysozyme standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. To construct the standard curve, plot the absorbance for Lysozyme standards (vertical axis) versus Lysozyme standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Standard	OD 450 nm	Concentration ng/mL
1	0.078	0
2	0.18	1.25
3	0.306	2.5
4	0.600	5
5	1.066	10
6	1.710	20
7	2.532	40

### Technical Support

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