# Lysozyme ELISA

**For the quantitative determination of lysozyme in human stool.**

**For Research Use Only. Not For Use In Diagnostic Procedures.**

**Catalog Number:** 30-LYSHU-E01

**Size:** 96 Wells

**Version:** 2022-02-02 – ALPCO 3.1

## INTENDED USE

The Lysozyme ELISA is intended for the quantitative determination of lysozyme in human stool samples. For Research Use Only. Not for use in diagnostic procedures.

## PRINCIPLE OF THE ASSAY

The assay utilizes the "sandwich" technique with two selected antibodies that recognize human lysozyme. Standards, controls, and diluted samples, which are assayed for human lysozyme, are added into the wells of a microplate coated with a high affinity anti-human lysozyme antibody. During the first incubation step, lysozyme is bound by the immobilized antibody. Then a peroxidase-conjugated anti-human lysozyme antibody is added into each microtiter well and a "sandwich" of capture antibody-human lysozyme-peroxidase conjugate is formed. Tetramethylbenzidine is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the enzymatic reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of lysozyme. A standard curve is generated using the values obtained from the standards. Lysozyme present in the samples is determined directly from this curve.

## MATERIALS SUPPLIED

| Component | Quantity | Preparation |
|---|---|---|
| Lysozyme Microplate (96 wells) | 12 x 8-well strips | Ready to use |
| Standards (1-5)<br>(0, 1.1, 3.3, 10, 30 ng/mL) | 5 x 1 mL vials | Ready to use |
| Control Levels 1 and 2 | 1 x 1 mL vial each | Ready to use |
| Wash Buffer Concentrate | 2 x 100 mL | 10x |
| Extraction Buffer Concentrate | 1 x 100 mL | 2.5x |
| Conjugate Concentrate | 1 x 50 µL | 1001x |
| Conjugate Dilution Buffer | 2 x 15 mL | Ready to use |
| TMB Substrate | 1 x 15 mL | Ready to use |
| Stop Solution | 1 x 15 mL | Ready to use |

## MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision pipettes for dispensing up to 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled/Deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 550 rpm, amplitude 2 mm
- Microplate reader
- Centrifuge (1,000 x g to 3,000 x g)
- Vortex for sample preparation
- Laboratory balance
- Foil to cover the microplate
- Timer

## PRECAUTIONS

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, all reagents should be treated as potentially infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. All materials derived from animal sources are bovine spongiform encephalopathy (BSE) negative. However, all materials should be treated as potentially infectious.
3. Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
4. The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
5. The 10x Wash Buffer concentrate contains surfactants which may cause severe eye irritation in case of eye contact.

Warning: Causes serious eye irritation
If in Eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists, get medical advice/ attention.

6. Avoid direct contact with skin.
7. This product is not for internal use.
8. Avoid eating, drinking, or smoking when using this product.
9. Do not pipette any reagents by mouth.
10. Reagents from this kit are lot-specific and must not be substituted.
11. Do not use reagents beyond the expiration date.
12. Variations to the test procedure are not recommended and may influence the test results.

## STORAGE CONDITIONS

The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label. The kit can be used up to 4 times within the expiry date stated on the label if stored properly.

## SAMPLE HANDLING

Stool samples are appropriate for use in this assay.

The sample stability is as follows:

**Raw stool:** 3 days at room temperature (15–30°C) and 2–8°C, and 3 months at -20°C.
**Stool extracts (1:100):** 1 day at room temperature (15–30°C), 5 days at 2–8°C or 7 days at -20°C, maximum 1 freeze-thaw cycle.

**Extraction of the Stool Sample:** 1X working extraction buffer is used as a sample extraction buffer. The following sample preparation is recommended:

1. The raw stool sample must be thawed. For particularly heterogeneous samples we recommend mechanical homogenization using an applicator, inoculation loop or similar device.
2. Fill the empty sample tube with 1.5 mL of 1X working extraction buffer before using it with the sample. Important: Allow the extraction buffer to reach room temperature. If using the EZ Extraction Device, the device is provided in a prefilled format and does not require any additional extraction buffer.
3. Unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into sample. The lower part

of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.
4. Shake the tube well until no stool sample remains in the notches. Important: Make sure the container has a maximally homogenous suspension after shaking. For more solid samples, soaking the sample in the tube with buffer for approximately 10 minutes improves results.
5. Allow sample to stand for approximately 10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
6. Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again. This will result in a dilution of 1:100 (**Dilution I**).

**Dilution I: 1:100**

**Dilution of Samples**

The supernatant of the sample preparation procedure (Dilution I) is diluted 1:2 in prepared wash buffer. For example:

125 µL supernatant (Dilution I) + 125 µL wash buffer, mix well = 1:2 dilution (Dilution II)
This results in a final dilution of **1:200**

For analysis, pipet 100 µL of Dilution II per well.

## REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used. To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. The kit can be used up to 4 times within the expiry date stated on the label. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.

***Wash Buffer Concentrate (10x)*** must be diluted with distilled water 1:10 before use (100 mL WASHBUF + 900 mL distilled water) and mixed well. Crystals may occur due to high salt concentration in the concentrate. The crystals must be dissolved at room temperature or in a water bath at 37°C before dilution of the buffer solution. The WASHBUF is stable at 2–8°C until the expiry date stated on the label. 1X working wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8°C for 1 month.

***Extraction Buffer Concentrate (2.5x)*** must be diluted with distilled water 1:2.5 before use (100 mL Extraction Buffer + 150 mL distilled water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals must be dissolved at 37°C in a water bath. The Extraction Buffer is stable at 2–8°C until the expiry date stated on the label. 1X working extraction buffer (1:2.5 diluted) can be stored in a closed flask at 2–8°C for four months.

***Conjugate Concentrate (1001x)*** must be diluted 1:1001 in conjugate dilution buffer (10 µL CONJ + 10 mL CONJBUF). The concentrate is stable at 2–8°C until the expiry date stated on the label. Diluted conjugate is not stable and cannot be stored.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2–8°C.

## QUALITY CONTROL

Control samples should be analyzed with each run. Results generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results

for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

## ASSAY PROCEDURE

**All reagents and microplate strips should be equilibrated to room temperature (15-30˚C) prior to use.** Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, controls, and samples should be run in duplicate. Store unused strips together with the desiccant bag in the closed aluminum packaging at 2–8°C.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact ALPCO.

1. Bring all reagents and samples to room temperature (15–30°C) and mix well.
2. Before use, wash the wells of the pre-coated microtiter plate 5 times with 250 µL 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
3. Add 100 µL of STD / SAMPLE / CTRL into the respective wells.
4. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker set to 550 rpm, orbit of 2 mm.
5. Aspirate the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6. Add 100 µL 1X working Conjugate into each well. *See Reagent Preparation for conjugate preparation instructions.*
7. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker set to 550 rpm, orbit of 2 mm.
8. Aspirate the contents of each well. Wash each well 5 times with 250 µL of wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9. Add 100 µL of Substrate into each well.
10. Incubate for 10–20 minutes at room temperature (15–30°C) in the dark.*
11. Add 100 µL of Stop Solution into each well and mix well.
12. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

*The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

## CALCULATION OF RESULTS

The following algorithms can be used alternatively to calculate the results. It is recommended to use the 4-parameter algorithm.

1. 4-parameter algorithm: It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).
2. Point-to-point calculation: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.
3. Spline algorithm: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility pairs of values should be examined before the automatic evaluation of the results. If this

option is not available with the program used, a review of the paired values should be done manually.

**Stool**

To obtain the lysozyme concentration of the samples, multiply by the **dilution factor of 200**. In case another dilution factor has been used, multiply the obtained result with the dilution factor used.

## LIMITATIONS

Samples with concentrations above the measurement range (see definition below) must be further diluted and retested. Please consider this higher dilution when calculating the results. Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

Highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

Analytical sensitivity* x sample dilution factor used.

*For analytical sensitivity of the assay, see Performance Characteristics.

## PERFORMANCE CHARACTERISTICS

### Analytical Sensitivity

The zero standard was measured 20 times. The detection limit was set as $B_0$ + 2 SD and estimated to be 0.5 ng/mL.

### Precision: Within run (intra-assay) variation

| | Sample 1 | Sample 2 |
|---|---|---|
| **Mean** | 6.7 ng/mL | 1.4 ng/mL |
| **CV (%)** | 7 | 10 |
| **n** | 18 | 18 |

### Precision: Between run (inter-assay) variation

| | Sample 1 | Sample 2 |
|---|---|---|
| **Mean** | 5.9 ng/mL | 1.7 ng/mL |
| **CV (%)** | 14 | 12 |
| **n** | 16 | 16 |

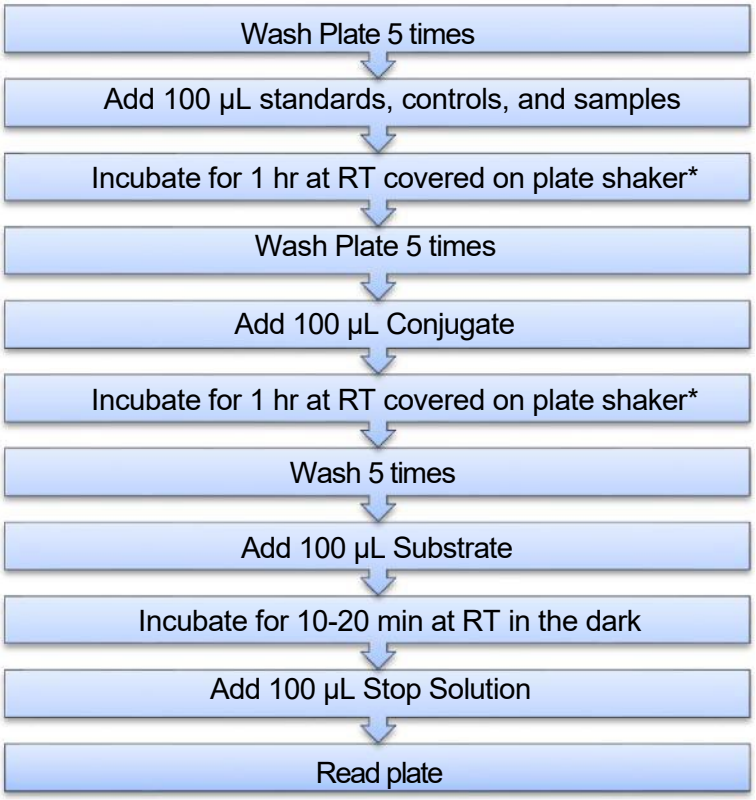
## TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is not recommended to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

## GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test.

### SHORT ASSAY PROTOCOL




**Total Incubation Time = 2 hours 20 minutes**

*The recommended plate shaker settings are 550 rpm with an orbit of 2 mm.

## SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running standards, controls, and up to 41 samples in duplicate.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Std 1 | Std 1 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| B | Std 2 | Std 2 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| C | Std 3 | Std 3 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| D | Std 4 | Std 4 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| E | Std 5 | Std 5 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| F | Ctrl1 | Ctrl 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| G | Ctrl 2 | Ctrl 2 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |
| H | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 | 41 | 41 |

Std= Standard, Ctrl = Control, Numbered wells = Samples