Biomedical Technologies Inc.

378 Page Street • Stoughton, MA 02072 USA • Phone: (781) 344-9942 Fax: (781) 341-1451 Web**:www.btiinc.com**

HUMAN LYSOZYME EIA KIT
Catalog No: BT-630

96 Well Tests Storage 4°C

***See Storage Exception on Page 2**

For the measurement of human lysozyme in serum, plasma, urine, tears, saliva, and other body fluids.

Introduction

Lysozyme (muramidase) hydrolyses principally the B-1,4 glucosidic linkages between n-acetylmuramic acid and n-acetylglucosamine occuring in the mucopeptide cell wall of some microorganisms. The enzyme has widespread distribution in animals and plants. In normal humans, relatively large concentrations of lysozymes are present in serum/plasma, amniotic fluid, saliva and tears with lessor quantities in urine, bile and cerebrospinal fluid. Elevated concentrations of urine and serum lysozyme have been reported in several human diseases and conditions including some leukemias, tuberculosis, megaloblastic anemias, acute bacterial infections, ulcerative colitis, severe renal insufficiency, pyelonephritis and nephrosis.

Principal of the Assay

This is a sandwich ELISA assay for human lysozyme. A monoclonal antibody specific for lysozyme is bound to polystyrene wells. After an incubation with sample, the plate is washed followed by an incubation with a second human lysozyme specific antibody (sheep polyclonal). Detection is achieved by a third incubation using a Horseradish Peroxidase conjugate of Donkey anti-Goat (sheep) IgG and subsequent enzyme assay. Concentration of human lysozyme is proportional to color development. Exact levels are obtained from a standard curve using purified human lysozyme.

References

1. Hankiewiz, J. and Swierczuk, E. 1974. Lysozymes in Human Body Fluids. Clinica Chemica Acta, 57: 205-209.
2. Meyor, K., Gelhorn, A., Prudden, J.F., et al. 1948. Lysozyme Activity in Ulcerative Alimentary Disease. American Journal of Medicine, 5: 496-502.
3. Prockup, D.J. and Davidson, W.D., 1964. A Study of Urinary and Serum Lysozyme in Patients with Renal Disease, New England Journal of Medicine, 270: 269.
4. Davis, C.S. April 5, 1971. Diagnostic Value of Muramidase. Laboratory Medicine, 51-54.

FOR RESEARCH USE ONLY.
NOT FOR USE IN HUMANS OR AS AN IN-VITRO DIAGNOSTIC. (REV. 09/04)

Reagents: Description and Preparation

Store all reagents at 4°C up to 6 months except as noted.

See Storage Exception

CAUTION: DO NOT USE AZIDE, OR AZIDE CONTAINING SAMPLES.

1. Phosphate-Saline Concentrate **BT-492**. One 100ml bottle. Transfer contents to a graduated cylinder, and bring volume up to 500ml with deionized water. Use this buffer for the preparation of standards, samples and for washing the plate.

2. Human Lysozyme Standard, **BT-631**. One vial, 1000ng lyophilized. Reconstitute with exactly 1ml deionized water. Use this stock solution for making working standards. Store the stock solution at -20°C up to 2 weeks.

3. Lysozyme Antiserum, **BT-632**. One 12ml vial.

4. Conjugate Buffer, **BT-633**. One 12ml vial.

5. Donkey anti-Goat IgG Peroxidase Conjugate, **BT-495**. Glycerol solution. One vial. ***Store at -20°C**. Dilute 1/800 (15ul for 12ml) using Conjugate Buffer. NOTE: only prepare enough solution for one day's use. Discard excess solution.

6. Peroxidase Substrate TMB, **BT-497**. One 6ml vial.

7. Hydrogen Peroxide Solution, **BT-498**. One 6ml vial.

8. Stop Solution, **BT-499**. One 12ml vial.

9. One 96 well plate (8 well removable strips) coated with a monoclonal human lysozyme antibody.

10. Human Lysozyme Control (Urine, Lyophilized), **BT-634**. Reconstitute with 0.5ml-1.0ml phosphate- saline buffer. Cap, mix end-over-end until the solids are dissolved. Store the solution at -20°C for one month.

Other Supplies Required

1. Elisa Plate Reader which can measure absorbance at 450nm.
2. Pipettes: micropipettes 5-1000ul.
3. A plate washer is recommended for washing.
4. A 37°C Incubator.
5. Deionized water.

Precautions

Some components of this kit contain isothiazolones (5ppm) as a preservative. Stop solution contains hydrochloric and phosphoric acids. Keep all materials away from the skin and eyes.

Sample Preparation

Collect samples in leak proof containers. Store serum (plasma), urine and body fluids (eg. saliva and tears) at 4°C for 2 days or 2 weeks at -20°C. Thaw and keep on ice until ready for use. **Caution: Samples must not contain azides**. Most samples require dilution with Phosphate-Saline Buffer: Urine, 1/10-1/50; serum (plasma), saliva, at least 1/2000; tears, approximately 1/10,000.

Range of Normal Values Reported

Serum (Plasma)	4-13ug/ml
Urine	0-2ug/ml
Saliva	4-13ug/ml
Tears	>300ug/ml

Values Observed at BTI

Urine	20-300ng/ml
Saliva	100ug-200ug/ml
Serum	3-10ug/ml

Standards

Prepare a set of standards from the 1000ng/ml stock in the range of 0.5 to 50ng/ml using diluted Phosphate-Saline Buffer. For example:

Standard #	ml of Std.	ml of Buffer	Concentration ng/ml
1.	.05 stock	0.95	50
2.	0.5 std. 1	0.5	25
3.	0.5 std. 2	0.5	12.5
4.	0.5 std. 3	0.5	6.25
5.	0.5 std. 4	0.5	3.125
6.	0.5 std. 5	0.5	1.56
7.	0.5 std. 6	0.5	0.78

Store the stock solution frozen (-20°C). Discard all working standards.

Assay Procedure

CAUTION: *KEEP AZIDES AWAY FROM ALL SOLUTIONS AND SAMPLES*

All Reagents must be at room temperature prior to use.

1. Prepare reagents, standards and samples as described on pages 2 and 3 respectively.

2. Remove microtiter plate from resealable bag. Strips not used should be removed from the frame, resealed in the bag and stored at 4°C for future use.

3. Pipet 100ul of wash buffer (Blank), standards, samples and controls into designated duplicate wells. Cover tightly with plastic seal and **incubate at room temperature for 2 hours.**

4. Aspirate wells completely and wash the plate 3 times with Phosphate-Saline wash buffer. Complete removal of wash buffer after each wash is important for good reproducibility. Add 100ul of the Lysozyme Antiserum to each well. Cover tightly, **incubate at room temperature for 1 hour**.

5. Wash as in step 4. Add 100ul of the diluted Donkey anti-Goat IgG Peroxidase to each well. **Incubate at room temperature for 1 hour.**

6. Mix one volume of TMB solution **(BT-497)** with one volume of Hydrogen Peroxide solution **(BT-498)** and put aside. Only mix an amount sufficient for the number of wells in use. Wash the plate as in step 4. Immediately add 100ul of substrate mix to all wells and **incubate at room temperature, in the dark for 15 minutes.**

7. Add 100ul of Stop Solution to all wells, swirl and measure absorbance at 450nm **within 15 minutes.**

Calculation of Results

Average duplicates for all determinations. Subtract the Blank from all average readings. Plot net optical density of the standards vs. log of the concentration of each. Draw the best curve. Obtain concentration of each unknown from this standard curve. Always generate a standard curve for each new assay.

Specifications

Sample size:	100ul
Assay time:	4.25hrs.
Sensitivity:	0.78ng/ml
Working range:	0.78-50ng/ml
Intraassay variation:	5.3%
Interassay variation:	7%
Recovery (urine):	105%

Typical Data (Do not use for determination of Unknowns)

ID	A 450nm	Average-Blank
Blank, 0ng/ml	.213	
Blank, 0ng/ml	.202	
0.78ng/ml	.393	
0.78ng/ml	.424	.201
1.5ng/ml	.656	
1.5ng/ml	.566	.404
3.12ng/ml	.889	
3.12ng/ml	.874	.674
6.25ng/ml	1.234	
6.25ng/ml	1.238	1.029
12.5ng/ml	1.778	
12.5ng/ml	1.739	1.555
25ng/ml	2.176	
25ng/ml	2.285	2.023
50ng/ml	2.400	
50ng/ml	2.546	2.266

Typical Standard Curve