Revised: 18–October–2001

EnzChek® Lysozyme Assay Kit (E-22013)

Quick Facts

Storage upon receipt:

- –20°C
- Dessiccate
- Protect from light

Ex/Em of Digestion Product: 494/518 nm

Introduction

The EnzChek® Lysozyme Assay Kit (E-22013) provides researchers with a sensitive assay to measure levels of lysozyme activity in solution. This fluorescence-based assay can detect lysozyme activity down to 20 U/mL (Figure 1). The assay measures lysozyme activity on *Micrococcus lysodeikticus* cell walls, which are labeled to such a degree that the fluorescence is quenched. Lysozyme action can relieve this quenching, yielding a dramatic increase in fluorescence that is proportional to lysozyme activity. The fluorescence increase can be measured using any spectrofluorometer, mini-fluorometer or fluorescence microplate reader that can detect fluorescein (Figure 2).

Lysozyme (muramidase) is a ubiquitous enzyme present in human serum, urine, tears, seminal fluid and milk. Lysozyme hydrolyzes β-(1-4)-glucosidic linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues present in the mucopolysaccharide cell well of a variety of microorganisms. Serum and urine lysozyme levels may be elevated in acute myelomonocytic leukemia (FAB-M4), chronic myelomonocytic leukemia (CMML) and chronic myelocytic leukemia (CML).[1] Increased serum lysozyme activity is also present in tuberculosis,[2] sarcoidosis,[2] megaloblastic anemias,[3] acute bacterial infections, ulcerative colitis[4] and Crohn's disease.[2,4] Elevated lysozyme levels in urine and serum occur during severe renal insufficiency,[5] renal transplant rejection,[6] urinary tract infections,[7] glomerulonephritis[5] and nephrosis.[5]

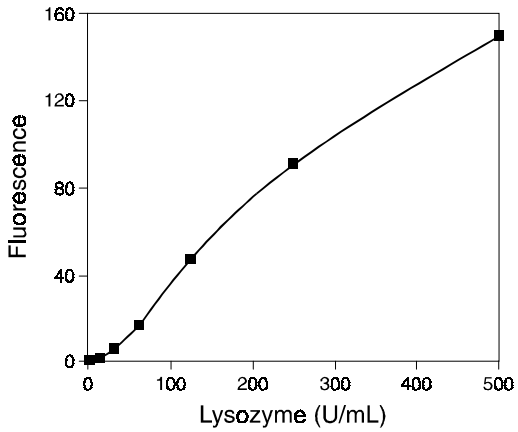

Figure 1. *Detection of lysozyme activity using the EnzChek Lysozyme Assay Kit. Increasing amounts of lysozyme were incubated with the DQ lysozyme substrate for 60 minutes at 37ºC. The fluorescence was measured in a fluorescence microplate reader using excitation/emission of ~485/530 nm. A background fluorescence of 20 fluorescence units was subtracted from each value.*

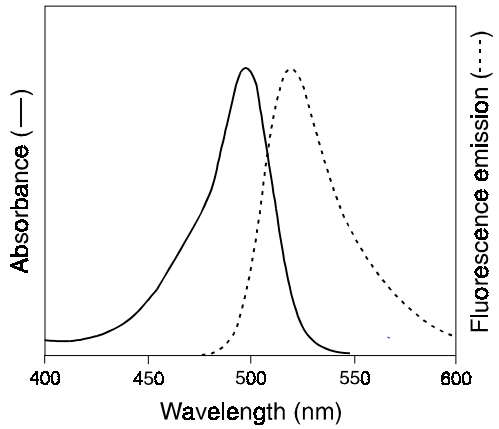

Figure 2. *Normalized absorption and fluorescence emission spectra of fluorescein.*

Materials

Kit Contents

- **DQ™ lysozyme substrate, fluorescein conjugate** (Component A), 1 mg of *Micrococcus lysodeikticus*, labeled with fluorescein
- **1X Reaction buffer** (Component B), 50 mL of 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 2 mM sodium azide as a preservative.
- **Lysozyme from chicken egg white** (Component C), 1000 U. One unit is defined as the amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25°C, using a suspension of *Micrococcus lysodeikticus* as the substrate.

Each kit provides sufficient reagents for 400 assays of 100 µL in a fluorescence microplate reader.

Storage and Handling

Upon receipt, the kit should be stored at -20°C, protected from light. For convenience, the reaction buffer may be stored unfrozen at 4°C. Allow the reagents to warm to room temperature before opening. When stored properly, these reagents are stable for at least six months.

Experimental Protocol

The following procedures are designed for use with a fluorescence multiwell microplate reader. For use with a standard fluorometer, volumes must be increased accordingly.

Reagent Preparation

1.1 Prepare a DQ lysozyme substrate stock suspension. Prepare a 1.0 mg/mL stock suspension of the DQ lysozyme substrate by suspending the contents of the vial (Component A) in 1.0 mL of deionized water (dH_2O). The DQ lysozyme substrate suspension can be stored at 4°C for at least one month. For long-term storage (up to six months), add 2 mM sodium azide and store at 4°C or freeze at -20°C in single-use aliquots. PROTECT FROM LIGHT. AVOID FREEZE–THAW CYCLES.

1.2 Prepare a 1000 U/mL lysozyme stock solution. Dissolve the contents of the vial (Component C) in 1 mL of dH_2O. The reconstituted lysozyme can be frozen in aliquots and stored at -20°C for at least six months without significant loss of activity. AVOID FREEZE–THAW CYCLES.

Lysozyme Activity Assay

The following protocol describes the assay of lysozyme activity in a total volume of 100 µL per microplate well. The kit provides sufficient material for ~400 assays.

2.1 Prepare a lysozyme standard curve. Fill 8 wells with 50 µL of 1X reaction buffer (Component B). Add 50 µL of the 1000 U/mL stock solution of lysozyme (prepared in step 1.2) to the first well, mix by pipetting, then transfer 50 µL to the second well. Repeat this process from one well to the next, except discard 50 µL from the mixture in the seventh well and add nothing to the eighth well. Thus, the lysozyme concentration will range 500 U/mL to 0 U/mL in the 50 µL volumes, for a range of 250 U/mL to 0 U/mL in the final 100 µL volumes.

2.2 Dilute the experimental samples. Dilute the lysozyme-containing, experimental samples in 1X reaction buffer (Component B). A volume of 50 µL will be used for each reaction and the concentration will be twofold lower in the final reaction mixture. A variable dilution will be required depending on the lysozyme activity present in the sample. In the first trial, the samples should be serially diluted to determine the optimal sample concentration.

2.3 Prepare a DQ lysozyme substrate working suspension. Prepare a 50 µg/mL working suspension of the DQ lysozyme substrate by diluting the 1 mg/mL stock suspension (prepared in step 1.1) 20-fold in 1X reaction buffer (Component B). A 50 µL volume will be used for each reaction. For example, to prepare enough working solution for 20 assays including the standard curve, add 50 µL of DQ lysozyme substrate stock suspension to 950 µL of 1X reaction buffer. Note that the final concentration of the DQ lysozyme working suspension will be twofold lower in the final reaction buffer.

2.4 Begin the reaction. Add 50 µL of the 50 µg/mL DQ lysozyme substrate working suspension (prepared in step 2.3) to each microplate well containing the experimental or the standard curve samples.

2.5 Incubate the reaction mixtures. Incubate the mixtures for 30 minutes or longer at 37°C, protected from light. Because the assay is continuous (not terminated), fluorescence can be measured at multiple time points to follow the kinetics of the reaction.

2.6 Measure fluorescence. Measure the fluorescence intensity of each reaction in a fluorescence microplate reader equipped with standard fluorescein filters. Digestion products from the DQ lysozyme substrate have absorption maxima at ~494 nm and fluorescence emission maxima at ~518 nm.

2.7 Correct for background fluorescence. Subtract the value derived from the no-enzyme control. Determine the lysozyme activity of the experimental samples from the standard curve.

References

1. Mod Pathol 7, 771 (1994); **2.** Acta Pathol Jpn 28, 689 (1978); **3.** N Engl J Med 277, 10 (1967); **4.** J Clin Pathol 36, 1312 (1983); **5.** Nephron 63, 423 (1993); **6.** Clin Chem 32, 1807 (1986); **7.** Toxicology 28, 347 (1983).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
E-22013	EnzChek® Lysozyme Assay Kit *400 assays*	1 kit

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