

Enzymatic Assay of STREPTOLYSIN O

PRINCIPLE:

Intact Red Blood Cells $\xrightarrow{\text{Streptolysin O}}$ Lysed Red Blood Cells

CONDITIONS: T = 37°C, pH = 7.4

METHOD: Visual determination of hemolytic activity.

REAGENTS:

- A. 10 mM Sodium Phosphate Buffer with 154 mM Sodium Chloride, pH 7.4 at 37°C (PBS-A)
(Prepare 50 mL in deionized water using Sodium Phosphate, Monobasic, Monohydrate, Prod. No. S-9638, Sodium Phosphate, Dibasic, Anhydrous, Prod. No. S-0876, and Sodium Chloride, Prod. No. S-9625.)
- B. 10 mM Sodium Phosphate Buffer with 103 mM Sodium Chloride, pH 7.4 at 37°C (PBS-B)
(Prepare 50 mL in deionized water using Sodium Phosphate, Monobasic, Monohydrate, Prod. No. S-9638, Sodium Phosphate, Dibasic, Anhydrous, Prod. No. S-0876, and Sodium Chloride, Prod. No. S-9625.)
- C. 10 mM Sodium Phosphate with 103 mM Sodium Chloride,
1 mg/mL Bovine Serum Albumin and 100 mM Dithioerythritol (Buffer I)
(Prepare 50 mL in Reagent B using Bovine Serum Albumin, Prod. No. A-4503, and Dithioerythritol, Prod. No. D-8255.)
- D. 2% (v/v) Suspension of human red blood cells. (2% RBCs)
(Prepare 5.0 mL by centrifuging human whole blood at low speed for 5 minutes. Decant the supernatant and gently resuspend the pellet in Reagent A in a ratio of 1:10. Centrifuge at low speed for 5 minutes. Repeat resuspension and centrifugation two more times. Dilute red blood cells to 2% (v/v) in Reagent B.)

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REAGENTS: (Continued)

- E. Streptolysin O Solution
(Immediately before use prepare a solution containing approximately 100,000 units per mL Streptolysin O in cold deionized water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into approximately 40 wells of a suitable microtiter plate.

		<u>Test</u>	<u>Blank</u>
Reagent C (Buffer I)	0.10	0.10	

Add 0.1 ml Reagent E to the first well. Incubate at 25°C for 15 minutes. Make serial dilutions of test solutions by consecutive transfers of 0.1 mL. Leave one well containing Reagent C only as a blank. Then add:

Reagent D (2 % RBCs)		0.05	0.05
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Incubate at 37°C for 30 minutes, then refrigerate for 30 minutes at 4°C. Record hemolysis for both test (serial dilutions) and blank.

CALCULATION:

$$\text{units/mg enzyme} = \frac{1}{\text{Mg Streptolysin O in well giving 50\% lysis}}$$

UNIT DEFINITION:

One unit will cause 50% lysis of 2% red blood cell suspensions in phosphate buffered saline, pH 7.4, after incubation at 37°C for 30 minutes.

FINAL ASSAY CONCENTRATION:

In a 0.25 mL reaction mix, the final concentrations are 10 mM Sodium Phosphate, 103 mM Sodium Chloride, 0.4% (v/v) Red Blood Cells, 0.4 mg/mL Bovine Serum Albumin, and 40 mM Dithioerythritol.

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NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.