



Product Information

STREPTOLYSIN O
from *Streptococcus pyogenes*
Sigma Prod. Nos. S5265 and S0149 (γ -irradiated)

CAS NUMBER: 98072-47-0

SYNONYM: SLO

PHYSICAL DESCRIPTION:

Appearance: White powder
Molecular weight: 69,000^{1,3,4}, although perhaps in error as 60,000.²
Isoelectric point: pI 6.0-6.4.¹

METHOD OF PREPARATION:

Sigma's precise procedure is proprietary; one reference provides general information.² Prior to lyophilization, S5265 and S0149 were dissolved in 10 mM Tris (1.2 mg/mL), 3 mM sodium azide (0.2 mg/mL), 5 mM EDTA (1.9 mg/mL) and 1 mM PMSF (0.2 mg/mL). Of this solution, 0.2 mL was dispensed per vial. Each vial will contain . 0.7 mg solid and 0.02 mg protein.³ S0149 has been gamma-irradiated to sterilize the product.

STORAGE / STABILITY AS SOLD:

Streptolysin O retains full activity for at least a year if stored dry at 2-8°C.³

SOLUBILITY / SOLUTION STABILITY:

SLO is soluble at 1 mg/ml cold deionized water.

SLO is readily oxidized in solution. It is recommended to use freshly prepared solutions, and to discard unused portions. Solution stability is improved in the presence of 20mM cysteine or 10mM dithiothreitol².

After reconstitution, the solution should be stored at -20 °C in aliquots. Avoid repeated freeze/thaw cycles. It is recommended to freeze solutions at a minimal concentration of 0.2 mg/ml.

GENERAL USAGE NOTES:

Streptolysin O is a single polypeptide chain devoid of amino sugars; it is an immunogenic oxygen-labile toxin which is reversibly activated by dithiothreitol.⁴ It is released into the extracellular medium along with other toxins (including streptolysin S) during the growth of most strains of group A and many strains of groups C and G streptococci.^{2,4}

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GENERAL USAGE NOTES: (continue)

It is differentiated from streptolysin S in that the latter is oxygen-stable, nonimmunogenic and only active when associated with a carrier protein.⁴

SLO is used for cell permeabilization or hemolysis; many hemolytic assays are reported for this application.³ The erythrocytes of different animal species have significantly different susceptibility to hemolysis by SLO.⁴

SLO was used to introduce oligonucleotides into cultured eukaryotic cells by permeabilization; mouse kidney cells were first trypsinized with 0.125% trypsin for 5 minutes, then pelleted, suspended at 50,000 cells in 0.25 mL buffer with SLO (0.2 units/mL SLO, in 137 mM NaCl, 100 mM PIPES pH 7.4, 5.6 mM glucose, 2.7 mM KCl, 2.7 mM EGTA, 1 mM ATP, 0.1% BSA). After 5 minutes, permeabilization was stopped with 5 mL DMEM/Ham's F-12 medium containing fetal bovine serum.⁵

SLO was used to permeabilize human T lymphocytes, after establishing an optimum concentration of 0.4 i.u. SLO/mL (>95% became permeable to trypan blue within 1 minute of incubation) with 5×10^7 cells/mL of buffer solution (120 mM KCl, 30 mM NaCl, 10 mM HEPES pH 7.2, 10 mM EGTA, 10 mM $MgCl_2$ and free calcium ion at 0 to 1 μ M).⁶

Extensive kinetic studies were performed on a partially purified SLO. Cholesterol was found to inhibit hemolytic activity when added before the incubation of SLO with rabbit erythrocytes, but not if added after the initiation of hemolysis.⁷

UNIT DEFINITION:

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

An international unit is related to the use of antibodies against SLO, defining the potency of an antibody. A Todd unit is the same as a "anti-streptolysin O titre" (ASOT) or the reciprocal of the serum dilution. Todd standardized SLO so that one volume of SLO solution just completely hemolyzed the same volume of a 5% rabbit blood cell suspension at 37°C in one hour. He then defined 1 unit of ASLO as the amount of serum (or commercial antibody) that neutralized 2.5 hemolytic doses of SLO. Sigma has not tested S5265 (or S0149) using rabbit erythrocytes or using antibodies.³

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REFERENCES:

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2. Alouf, J.E. and Geoffroy, C., *Methods in Enzymology*, 165, 52 (1988).
3. Sigma quality control or production records.
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5. Barry, E. et al., *Biotechniques*, 15, 1016 (1993).
6. Graves, J.D. et al., *Biochem. J.*, 265, 407-413 (1990).
7. Kanbayashi, Y. et al., *J. Biochem.*, 71, 227-237 (1972).

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