Streptolysin O (Hemolytic *streptococcus*), functional

01-531 20 µg 01-531-5 5 x 20 µg

Storage: Ship at 4℃ or -20℃, and store at -20℃ (For longer period, years, store at -80℃)

Product: Recombinant streptolysin O. Functional in membrane pore formation to introduce molecules into living animal cells. The product was highly purified from *E.coli* over-expressing SLO of Group C hemolytic *streptococci* (His6-tagged to the signal peptide removed N-terminal of SLO). The specific activity is as high as 600,000-1,000,000 hemolytic units (HU) /mg (depending on Lot).

Applications

1) Functional studies

2).Reagent for membrane pore formation to introduce small-to-macromolecules into living cells (For protocol refer to or other references; Walev I *et al* "Delivery of proteins into living cells by reversible membrane permeabilization with streptolysin-O." *PNAS* **98**: 3185-3190 (2001) PMID: 11248053)

3).Antigen for the measurement of anti-streptolysin O antibody (ASO) (diagnostic reagent), ELISA

4) Western blotting, Dot blotting,

5) Immuno-chromatography

6) SDS-PAGE

Measurement of the activity: Definition of 1HU is activation of 50% hemolysis by incubating 3% sheep red blood cells at 37℃ for 30 min.

Purity: Over 98% by SDS-PAGE (see Fig.1)

Form: 1 mg/ml in PBS (-), 1 mM DTT, 50% glycerol, sterilized by filtration. No additive nor carrier protein.

Inactivated SLO can be reactivated by thiol reagents such as 20 mM cysteine or 10 mM DTT (Palmer M. *Toxicon* **39**: 1681-1689 (2001) PMID: 11595631)

Background: Streptolysin O (SLO) is a membrane-damaging extracellular toxin produced by hemolytic *streptococci*. The membrane-damaging activity is measured by hemolysis of red-blood cells. SLO is easily inactivated in the presence of oxygen but can be reactivated by thiol compounds, so it is also called thiol-activated cytolysin . SLO is produced not only by Group A hemolytic *streptococci* but also by Group C and Group G strains. The amino acid sequences are highly conserved among them and their homology is over 98%.

Data Link : UniProtKB Q54114 ((TACY_STREQ)

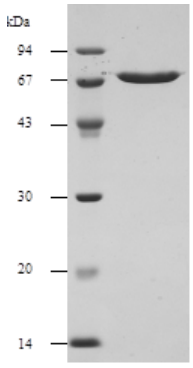

Fig1. Purified SLO analysed by SDS-PAGE.
The SLO has molecular mass of 60.4 kDa.
This product has molecular mass of 64.5 kDa

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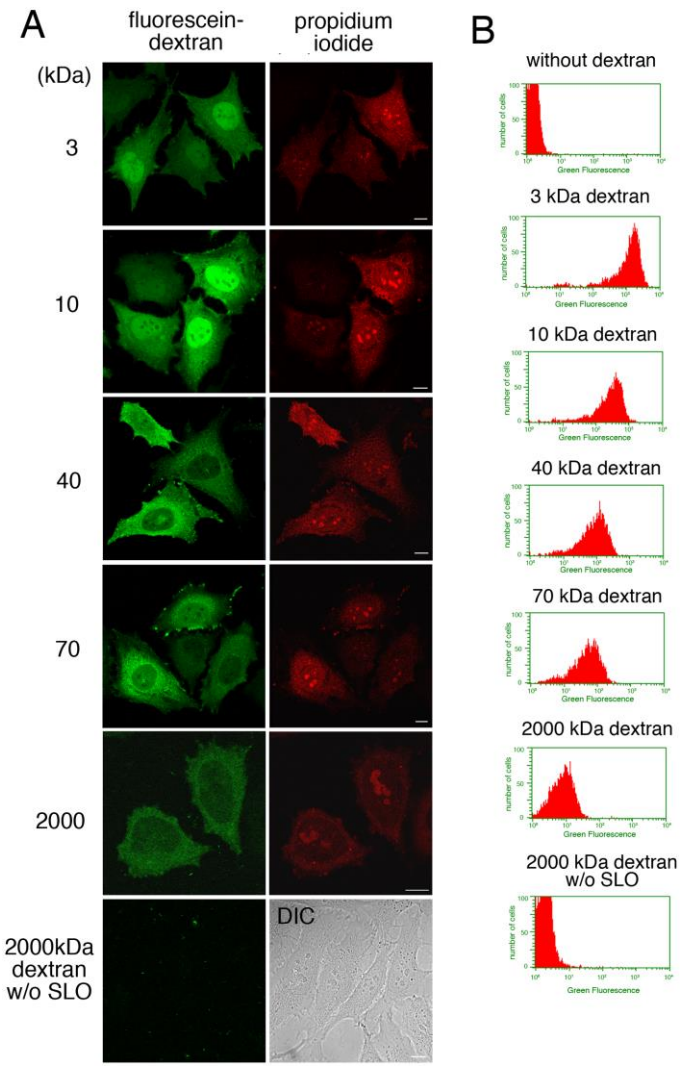

Fig.2 Introduction of fluorescein dextran of different molecular weights into resealed cells. A. HeLa cells were incubated with or without (2000 kDa dextran w/o SLO) 0.13 μg/ml SLO on ice for 5 min. After wash with PBS three times, the cells were further with transport buffer containing propidium iodide at 32°C for 5 min. Semi-intact HeLa cells were incubated with 1.5 mg/ml L5178Y cytosol, an ATP regenerating system, GTP, glucose, and 100 μg/ml fluorescein-dextran of 3, 10, 40, 70, or 2000 kDa at 32°C for 15 min, and then were resealed by treatment with 1 mM $CaCl_2$ at 32°C for 5 min. After incubation with DMEM supplemented with FCS for 30 min, the cells were observed by confocal microscopy. Since the cells without SLO treatment did not contain the fluorescence of propidium iodide, differential interference contrast (DIC) image was shown. Bar = 10 μm. B. HeLa cells were treated as described in A, were trypsinized, and were subjected to flowcytometry. The histograms of fluorescein fluorescence of dextran with different molecular weight in PI-positive cells were shown. **Data from Kano F. et al, PLoS One. 2012;7(8):e44127.**

References : This product has been used in the following publications.

1. Maeda, Y. et al. GPHR is a novel anion channel critical for acidification and functions of the Golgi apparatus. Nat. Cell Biol. 10: 1135-45 (2008) PMID: 18794847 **Permeabilization of cells.**
2. Furukawa K. et al. Reduction-triggered fluorescent amplification probe for the detection ofendogenous RNAs in living human cells. Bioconjug Chem. 2009 May

20;20(5):1026-36. PMID: 19374406 **Introduction of probes for RNA into permeabilized human HL cells**

3. Thiery J. et al. Perforin activates clathrin- and dynamin-dependent endocytosis, which is required for plasma membrane repair and delivery of granzyme B for granzyme-mediated apoptosis. Blood 2010 115:1582-1593. PMID: 20038786 **Promotion of endocytosis**
4. Kano F. et al. Hydrogen peroxide depletes phosphatidylinositol-3-phosphate from endosomes in a p38 MAPK-dependent manner and perturbs endocytosis. Biochim Biophys Acta. 2011 May;1813(5):784-801. PMID: 21277337. **Permeabilization of HeLa cells.**
5. Potez S. et al. Tailored protection against plasmalemmal injury by annexins with different Ca2+ sensitivities. J Biol Chem. 2011 May 20;286(20):17982-91. PMID: 21454475 **Permeabilization of HEK cells.**
6. Kano F. et al. A resealed-cell system for analyzing pathogenic intracellular events: perturbation **of** endocytic pathways under diabetic conditions. PLoS One. 2012;7(8):e44127. PMID: 22952896 **Introduction of molecules into HeLa cells.**
7. Imai A. et al. MADD/DENN/Rab3GEP functions as a guanine nucleotide exchange factor for Rab27 during granule exocytosis of rat parotid acinar cells. Arch Biochem Biophys. 2013 Aug 1;536(1):31-7. PMID: 23702376. **Introduction of antibody into cell**
8. Gao N and Lehrman MA. Mannose-6-Phosphate: A Regulator of LLO Destruction. : Inka Brockhausen (ed.), Glycosyltransferases: Methods and Protocols, 2013, Springer, Methods in Molecular Biology, vol. 1022, DOI 10.1007/978- Inka Brockhausen (ed.), Glycosyltransferases: 1-62703-465-4_20. Link:.springer.com/protocol/10.1007 **Introduction of mannose-6-phosphate into living cells. (The authors specifically recommend BioAcdemia streptolysin O for cell permeabilization)**
9. Matsuto M et al. Reconstitution of the targeting of Rab6A to the Golgi apparatus in semi-intact HeLa cells: A role of BICD2 in stabilizing Rab6A on Golgi membranes and a concerted role of Rab6A/BICD2 interactions in Golgi-to-ER retrograde transport. Biochim Biophys Acta. 2015 Oct;1853(10 Pt A):2592-609. PMID: 25962623 **Introduction of protein (Rab6A) into permeabilized Hela cells.**
10. Yasuga H. et al. Logic gate operation by DNA translocation through biological nanopores. PLoS One. 2016 Feb 18;11(2):e0149667. PMID: 26890568 **Nanopore formation in bilayer lipid menmbrae.**
11. Ojima K. et al. Myosin substitution rate is affected by the amount of cytosolic myosin in cultured muscle cells. Anim Sci J. 2017 Nov;88(11):1788-1793. PMID:28631391 **Permeabilization of cells**
12. Kano F. et al. Establishment and phenotyping of disease model cells created by cell-resealing technique. Sci Rep. 2017 Nov 9;7(1):15167. PMID:2912317011. **Reversible permeabilization of plasma membrane**
13. Watanabe H. et al. Analysis of Pore Formation and Protein Translocation Using Large Biological Nanopores. Anal Chem. 2017 Nov 7;89(21):11269-11277. PMID:28980803. **Permeabilization for protein translocation**

Material Safety Data Sheet Revised on August 9, 2019

1. IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifiers

Product name: Streptolysin O, functional recombinant protein

Product code: 01-531

1.2 Relevant identified uses of the substance or mixture and uses advised against For research use only and not for use in diagnosis or in human.

1.3 Details of the supplier of the safety data sheet

Responsible Party

Company Name: BioAcademia Inc.

Address: North Buiding, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Tel: 81-6-6877-2335 Fax: 81-6-6877-2336

E-mail: Info@bioacademia.co.jp

1.4 Emergency telephone number: 81-6-6877-2335

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008. This substance is not classified as dangerous according to Directive 67/548/EEC. 2.2 Label elements The product does not need to be labelled in accordance with EC directives or respective national laws.

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2.3 Other hazards – none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Composition of product: Streptolysin O (0.1%, CAS No. N/A), Glycerol (50%, CAS No. 56-81-5), x1 Phosphate Buffered Saline (KH_2PO_4 0.021%, CAS No. 7778-77-0 : NaCl 0.9%,CAS No. 7647-14-5: Na_2HPO_4 0.038%, CAS No 7558-79-4.), Dithiothreitol (0.015%,CAS No. 3483-12-3), H_2O (49%,CAS No. 7732-18-5)

4. FIRST AID MEASURES

4.1 Ingestion: Wash out with large amount of water. When swallowed, get medical attention if any discomfort arises.

4.2 Eye contact: Wash with large amounts of water while lifting eye lids. Call medical doctor if irritation continues.

4.3 Skin contact: Wash off with soap and plenty of water.

4.4 Spill release: Wear glove and sweep up the spill and then wash spill site. All the contaminants should be autoclaved at 121℃ for 20 min before disposal.

5.Handling and Storage

5.1 Handling and Storage Precautions:BIOHAZARD. DO NOT USE IF SKIN IS CUT OR SCRATCHED.

5.2 Other Precautions:CAUTION: SUBSTANCE NOT YET FULLY TESTED.

6. Toxicological Information

6.1 Target: Cholesterol on human and animal cell membrane

6.2 Health Hazards: May be fatal if large amount enters bloodstream.

6.2 LD50 - Lethal dose (50 percent kill) intravenous,

Rabbit, 1500 ng/kg (Ref :PHTHDT Pharmacology and Therapeutics. (Pergamon Press Ltd.,Headington Hill Hall, Oxford OX3 0BW, UK) Vol.(Issue) 11, Page 661,1981)

Guinea pig, 12 ug/kg (Ref: BICMBE Biochimie. (SPPIF, B.P.22, F-41353 Vineuil, France, Vol.(Issue)55,Page 1187, 1973)

Toxicity is much less when introduced via other routes of entry like Interdermal injection

7. Exposure Controls/Personal Protection

7.1 Protective Gloves:COMPATIBLE CHEMICAL-RESISTANT GLOVES.

7.2 Eye Protection:ANSI APPROVED CHEMICAL WORKERS GOGGLES .

7.3 Other Protective Equipment:EYE WASH AND DELUGE SHOWER MEETING ANSI DESIGN CRITERIA .

7.3 Work Hygienic Practices:WASH THOROUGHLY AFTER HANDLING.

8. Disposal Considerations

Waste Disposal Methods: Autoclave the waste at 121℃ for 20 min.

9. Regulatory Information

Federal Regulatory Information:EUROPEAN INFORMATION: CAUTION: SUBSTANCE

NOT YET FULLY TESTED.

Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.