PRODUCT INFORMATION

Perfringolysin O (C459A mutant; *C. perfringens*, recombinant)

Item No. 42617

Overview and Properties

Synonyms:	PFO, PFO^{C459A}, rPFO^{C459A}, Thiol-activated Cytolysin, θ Toxin
Source:	Active recombinant *C. perfringens* N-terminal His-tagged PFO^{C459A} expressed in *E. coli*
Amino Acids:	29-500
Uniprot No.:	P0C2E9
Molecular Weight:	54.5 kDa
Storage:	-80°C (as supplied)
Stability:	≥1 year
Purity:	≥90% estimated by SDS-PAGE
Supplied in:	20 mM potassium phosphate pH 8.0, with 100 mM sodium chloride, 10% glycerol, and 5 mM β-mercaptoethanol
Protein Concentration:	***batch specific*** mg/ml
Specific Activity:	See images

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Flow Cytometry Assay Application

Perfringolysin O (C459A) (PFO^{C459A}) is a toxin secreted by *C. perfringens* that permeabilizes the plasma membrane in live cells. This membrane disruption can be measured *via* inclusion or exclusion of DAPI (Item Nos. 40796 | 14285) in flow cytometry experiments. Intact cells will not contain DAPI but cells permeabilized by PFO will be DAPI-positive.

Materials Needed But Not Supplied

1. A flow cytometer capable of running forward scatter/side scatter (FSC/SSC) in parallel with a DAPI^{+} capture field
2. Adjustable pipettes; repeating pipettor recommended
3. 96-well V-bottom tissue culture plate
4. Triton™ X-100 (10% v/v)
5. DAPI (100X)
6. Materials needed for cell culture

Reagent Preparation

1. Triton™ X-100 (10% v/v): Prepare a 0.1 working solution
2. PFO^{C459A} stock solutions: Prepare 100X working solutions for PFO^{C459A} at 1, 10, and 100 nM final tissue culture plate concentrations using an appropriate complete cell culture medium
3. Running buffer: Add BSA into PBS to a final concentration of 1% (w/v) BSA

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Sample Assay Protocol

1. Seed THP-1 cells (~5×10^5 cells/ml) or other cells of interest in a 96-well V-bottom plate at 200 µl/well in an appropriate complete cell culture medium. Ensure the number of seeded wells matches the number of wells required to assess unstained, negativecontrol (untreated), positive control, PFO^{C459A} 1 nM, PFO^{C459A} 10 nM, and PFO^{C459A} 100 nM wells in triplicate.
2. Add 2 µl of DAPI (100X) to all wells except the wells designated as unstained.
3. Add reagents to all other groups:
   a. Positive control: 2 µl of diluted Triton™ X-100 (10% v/v)
   b. PFO^{C459A} 1 nM: 2 µl of 100X working solution
   c. PFO^{C459A} 10 nM: 2 µl of 100X working solution
   d. PFO^{C459A} 100 nM: 2 µl of 100X working solution
4. Mix gently and incubate for 10 min at room temperature protected from light.
5. Spin at 500 x g for 5 minutes, then empty the wells by flicking it out of the plate and into a waste container. Resuspend the cells with 150 µl of running buffer.
6. Acquire by flow cytometry: Use the unstained FSC/SSC to set the voltages for the cell population and check DAPI background. Use the DAPI$^+$ population to set the acquisition voltage.

Expected Output:

Cells treated with PFO^{C459A} will demonstrate a dose-dependent increase in DAPI signal compared to untreated cells, which will be ≤10% DAPI$^+$ cells in the whole population. These results will guide the amount of enzyme needed for future assays, including mitochondrial related assays.

This product also can be used in Mitochondrial Oxygen Consumption Assay.

Sample Results

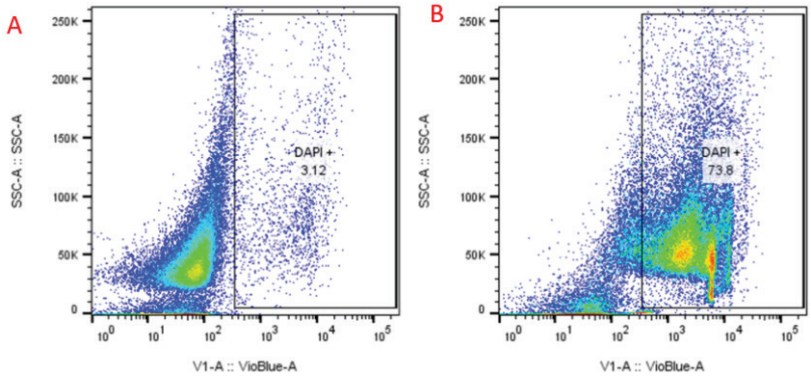

Figure 1. DAPI signal in PFO^{C459A}-permeabilized THP-1 cells. A) Untreated THP-1 cells (negative control) exclude DAPI. B) THP-1 cells incubated with PFO^{C459A} 10 nM for 10 minutes demonstrate a significantly increased DAPI signal due to permeabilization of the plasma membrane.

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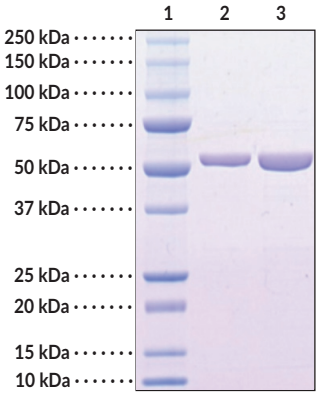
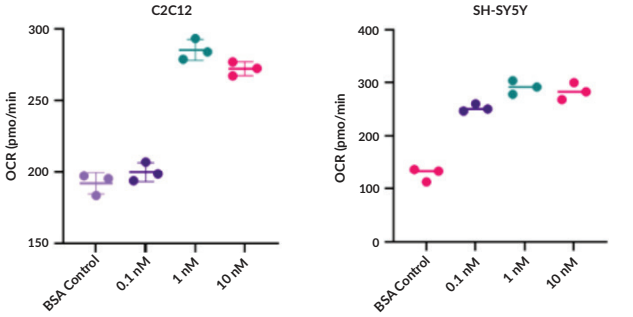
Images



Lane 1: MW Markers
Lane 2: Perfringolysin O (2 µg)
Lane 3: Perfringolysin O (4 µg)

SDS-PAGE Analysis of Perfringolysin O.



Mitochondrial oxygen consumption in Perfringolysin O C459A (PFO^{C459A})-permeabilized cells. C2C12 and SH-SY5Y cells (2x10^4 cells/well) were permeabilized with 0.1-10 nM PFO^{C459A} and oxygen consumption rates were measured *via* Seahorse™ respirometry in mitochondrial respiration medium: 220 mM sucrose, 70 mM mannitol, 10 nM potassium phosphate, 5 mM magnesium chloride, 2 mM HEPES, 1 mM EGTA, 10 mM glutamate, 2 mM malate, 4 mM ADP, and 0.2% BSA at pH 7.4.

Description

Perfringolysin O (PFO) is a protein member of the cholesterol-dependent cytolysin (CDC) family.[1,2] It is composed of four β-sheet-rich domains and a C-terminal undecapeptide motif.[1] PFO is a toxin secreted by *C. perfringens* and induces cholesterol-dependent cytolytic β-barrel pore formation in the plasma membrane of host cells, such as erythrocytes and leukocytes.[1,3] PFO containing a cysteine-to-alanine substitution at position 459 (PFO^{C459A}) has a higher cholesterol membrane concentration threshold for pore formation compared to wild-type PFO and has been used as a cell membrane permeability agent in mitochondrial and metabolic studies.[2,4] Cayman's Perfringolysin O (C459A mutant; *C. perfringens*, recombinant) protein can be used for cell-based assays.

References

1. Verherstraeten, S., Goossens, E., Valgaeren, B., *et al.* Perfringolysin O: The underrated *Clostridium perfringens* toxin? *Toxins (Basel)* **7(5)**, 1702-1721 (2015).
2. Divakaruni, A.S., Wiley, S.E., Rogers, G.W., *et al.* Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier. *Proc. Natl. Acad. Sci. USA* **110(14)**, 5422-5427 (2013).
3. Awad, M.M., Ellemor, D.M., Boyd, R.L., *et al.* Synergistic effects of alpha-toxin and perfringolysin O in *Clostridium perfringens*-mediated gas gangrene. *Infect. Immun.* **69(12)**, 7904-7910 (2001).
4. Yang, K., Doan, M.T., Stiles, L., *et al.* Measuring CPT-1-mediated respiration in permeabilized cells and isolated mitochondria. *STAR Protoc.* **2(3)**, 100687 (2021).

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