

PRODUCT DATA SHEET

Product: Perforin (natural human)

Cat. No.: EN-008 (1 µg)

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Origin:

Cytotoxic granules of YT cells

Molecular Weight:

~70 kDa

Purification:

Purified from cytotoxic granules by solubilization in 1M NaCl (Sodium acetate buffer) followed by hydrophobic interaction chromatography.

Format:

Liquid in 0.15M NaCl with protein stabilizer. Product provided as 1 µg vial of Perforin (PFN) and 1 mL vial of PFN Dilution Buffer.

Storage:

The stock perforin may be diluted to desired concentration up to a 10-fold level with the provided dilution buffer. The diluted perforin may then be sub-aliquoted and frozen (-80°C). The frozen diluted perforin tolerates two freeze-thaw cycles and is stable for up to 3 months. After thaw the diluted perforin is stable at 4°C for approximately 12 hrs.

Specific Activity:

10.3 permeabilizing units (PU) (Jurkat) / µg. Cells (1×10^6 /mL) are incubated for 15 min at room temperature with various concentrations of perforin and then stained with Propidium iodide (PI). Permeabilized (PI-positive) and non-permeabilized cells are quantified by FACS (or UV light microscopy). One PU is defined as the amount of PFN (ng) that produces 50% PI+ cells in the sample. The capacity of perforin to permeabilize nucleated cells varies substantially. The investigator must identify the concentration that provides the desired level of permeabilization for the cell line in question.

Handling:

For maximum product recovery after thawing, centrifuge the vial before opening the cap.

Protocol: For intracellular delivery of proteins by human PFN

Both primary and transformed lines are permeabilized by the perforin (data available on request). However, the concentration of perforin (ng/mL) required to produce 50% permeabilized cells varies substantially according to cell type (e.g., lymphoid, epithelial, mesenchymal) and whether cells are suspended or adherent. To determine overall activity, perforin has been titrated against the model cell line Jurkat. Consumption of the perforin will vary according to the sensitivity of the cell line the user plans to examine. **PFN should be pre-titrated against the desired cell line to identify the minimally permeabilizing concentration for protein delivery** (see below). This concentration will allow effective delivery of the protein without producing excessive background necrosis and represent the nanograms that achieve less than 10 – 20% permeabilization of the target cell in question.

A. Materials

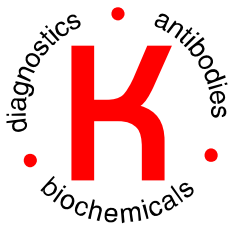
- Microfuge tubes (1.5 mL)
- Calcium Stock: 100 mM CaCl₂ in water
- Ca⁺⁺ HEPES buffer: 20 mM HEPES, 150 mM NaCl, 2.5 mM CaCl₂ (pH 7.4)
- FA-Free BSA
- HEPES/1% BSA: 1% BSA solubilized in HEPES buffer lacking Ca⁺⁺
- PFN Dilution Buffer: Shipped with PFN.

B. Dilution and storage of PFN

The stock PFN may be diluted to the desired concentration up to a 10-fold level with the provided dilution buffer. The diluted PFN may then be sub-aliquoted and frozen (-80°C). The frozen dilute PFN tolerates two freeze-thaw cycles and is stable for up to 3 months. After thaw the diluted PFN is stable at 4°C for approximately 12 hrs.

C. Pre-titration of PFN against desired cells

Immediately before the planned experiment, PFN should be titrated to identify concentration that minimally permeabilizes targets. Incubate desired cells with increasing concentrations of PFN for 15 min then determine percentage of cells that have undergone permeabilization by Trypan Blue or Propidium Iodide stain.



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- Step 1** Wash cells twice in Ca⁺⁺ HEPES buffer to remove serum from cell mixture and re-suspend at density of 1×10^6 / 50 µL in same buffer.
- Step 2** For each sample, dilute PFN to twice the desired final concentration in 50 µL of HEPES / 1% BSA to microfuge tubes.
- Step 3** Add 50 µL of media control or dilute PFN to 50 µL of cells in microfuge tubes.
- Step 4** Incubate at 37°C for 15 min - periodic mixing is not required.
- Step 5** Perform readout to assess permeabilization.

D. Experimental Protocol for PFN mediated protein delivery

- Step 1** Wash cells twice in Ca⁺⁺ HEPES buffer to remove serum from cell mixture and re-suspend at density of 1×10^6 / 50 µL in same buffer.

- Step 2** Add Granzyme B (1 µg/mL); generally 1-2 µL of stock is sufficient.
- Step 3** For desired samples, dilute PFN to twice the desired final concentration in 50 µL HEPES/1% BSA to microfuge tubes.
- Step 4** Add 50 µL of media control or dilute PFN to 50 µL of cells in microfuge tubes.
- Step 5** Incubate at 37°C for desired time - periodic mixing is not required.
- Step 6** After incubation, perform readout.

Limitations:

For *in vitro* research use only. Not for use in diagnostics or in humans.

Warranty:

No warranties, expressed or implied, are made regarding the use of this product. KAMIYA BIOMEDICAL COMPANY is not liable for any damage, personal injury, or economic loss caused by this product.