GP91phox

Product specification

Acronym: gp91phox (Nox2 subunit)
Class: Enzyme
Origin: Human
Molecular weight: 55 kDa
Application: Screening & display technologies.

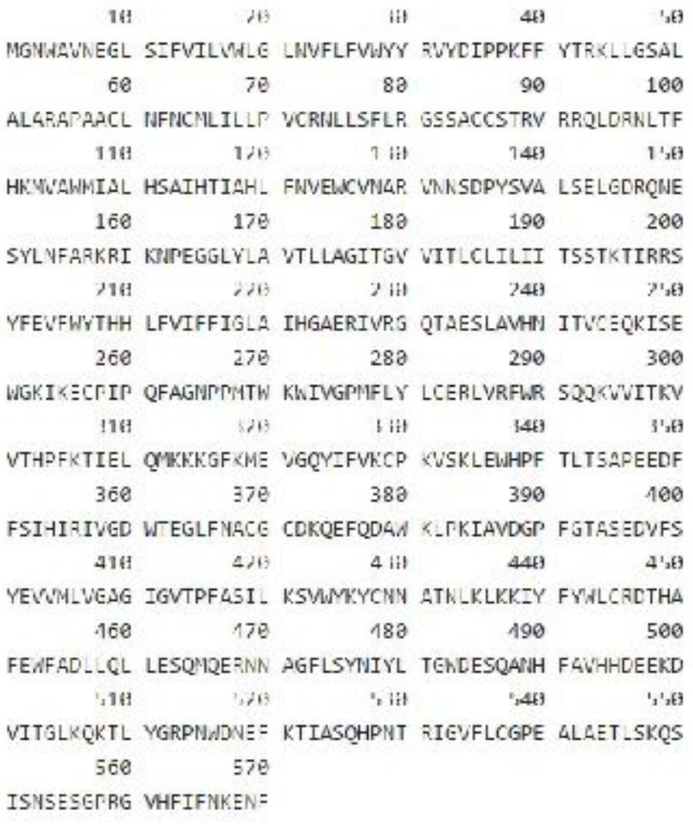
Purity: >50%
Activity: Proven
Length: Full Length
TMD: 2
Biological function: Phagocyte NADPH oxidase subunit

Product description

Critical component of the membrane-bound oxidase of phagocytes that generates superoxide. It is the terminal component of a respiratory chain that transfers single electrons from cytoplasmic NADPH across the plasma membrane to molecular oxygen on the exterior. Also functions as a voltage-gated proton channel that mediates the H+ currents of resting phagocytes. It participates in the regulation of cellular pH and is blocked by zinc.

Protein Source: gp91phox wild type protein

Fig.1: AA sequence of gp91phox protein



Affinity Tag: Histidine tag fused to the N-terminal end of the protein.

Production conditions: gp91 Phox is expressed in a cell-free expression system in the presence of lipid vesicles. 100 µg can be produced and qualified in about 1 week.

Quality analysis

Purity: Typically > 50% as determined by SDS-Page and Coomassie Blue staining.

Purification procedure: As standard, gp91 Phox proteoliposomes are purified on a sucrose gradient. Further purification steps can be added if required.

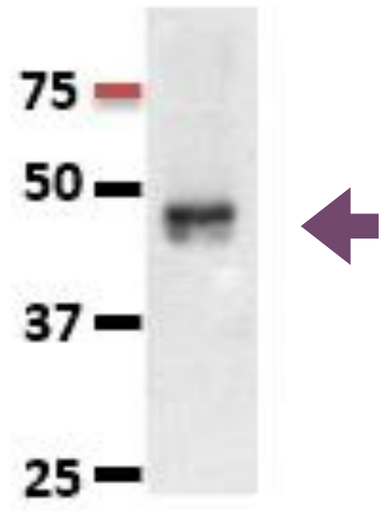


Fig.2: Proteoliposome gp91Phox after purification (Western blot identification).

Assessment of functionality

Cell-free expression systems provide a real alternative for membrane protein expression, enabling the study of structure and function of membrane proteins.

Methods:

The Human p22 Phox protein was coexpressed with the Nox2 protein in Synthelis' cell-free system in the presence of liposomes to obtain proteoliposomes in a one-step reaction. The nitroblue tetrazolium (NBT) assay was used to assess the NADPH oxidase activity of PLs NOX2/p22phox.
The nitroblue tetrazolium (NBT) test is an indirect marker of the oxygen-depend bactericidal activity of the macrophages. NBT is a dye with low reduction potential and performs intensively stained products–formazanes. NBT is easily phagotized by cells and is reduced to formazane inside mitochondrium.

Results:

The ability of these proteoliposomes to restore the *in cellulo* NADPH oxidase activity in the ROS-deficient macrophages has been analysed. NBT test performed on X0-CGD macrophages treated with NOX2/p22phox liposomes during 24h showed a blue precipitate of formazan, revealing the production of ROS upon PMA (Phorbol Myristate Aetate) stimulation.

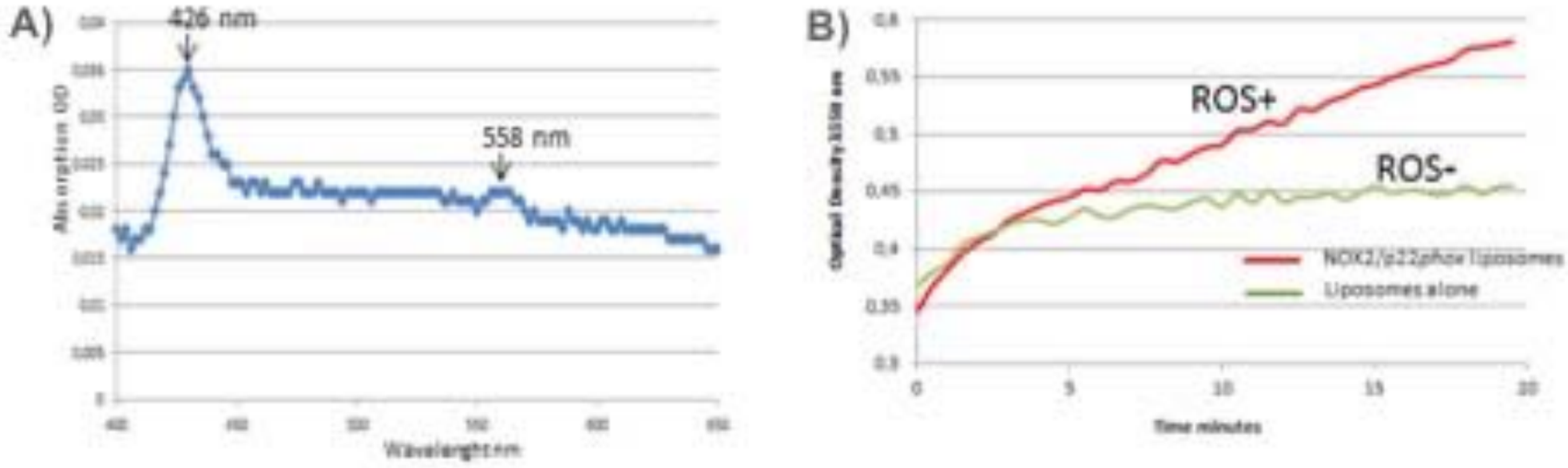


Fig.3: Analysis of Nox2/p22phox liposomes functionality. A) Differential spectrum of purified cytochromeb558 (Nox2/p22phox) in lipid vector. B) In vitro NADPH oxidase activity (in vitro assay).

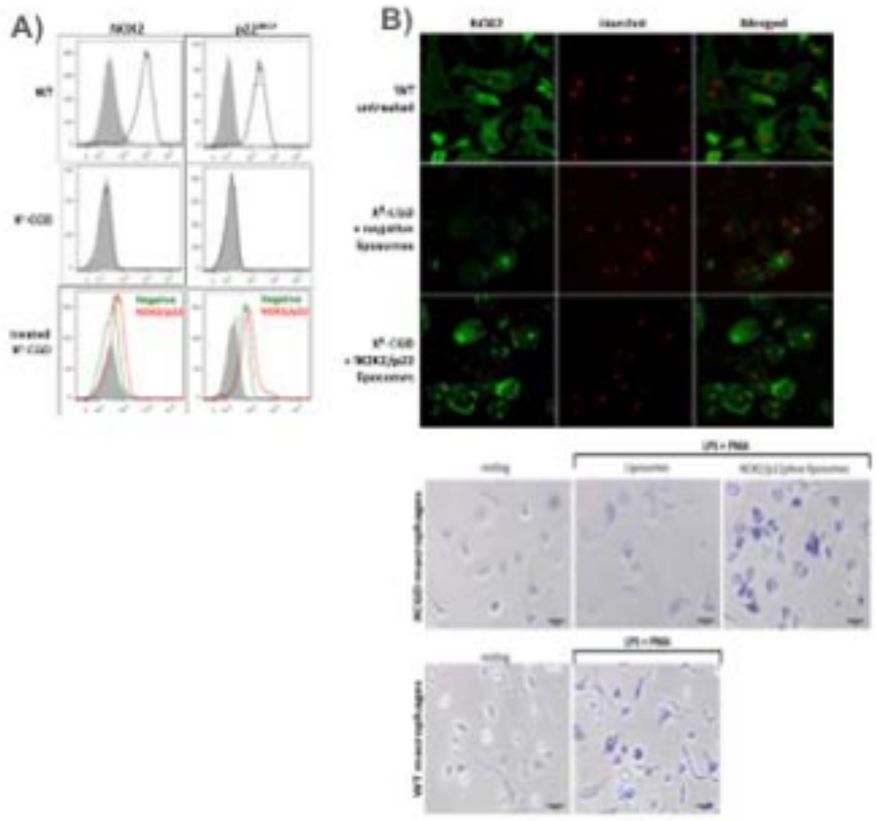


Fig.4: Analysis of in cellulo incorporation of NOX2/p22phox liposomes in the membranes of iPSCs-derived macrophages.

A-Flow cytometry analysis of NOX2 and p22phox expression using monoclonal antibodies (black curve) in WT and X0-CGD macrophages, and X0-CGD macrophages treated for 24h with NOX2/p22phox (red curve) or negative (green curve) liposomes. Isotype controls are represented by gray-filled curves. B- Confocal microscopy showing the staining of NOX2 protein with 7D5 antibody and FITC-conjugated secondary antibody (green) in WT and X0-CGD macrophages treated for 24h with NOX2/p22phox or negative liposomes. Nuclei were counterstained with Hoechst in red.

Formulation

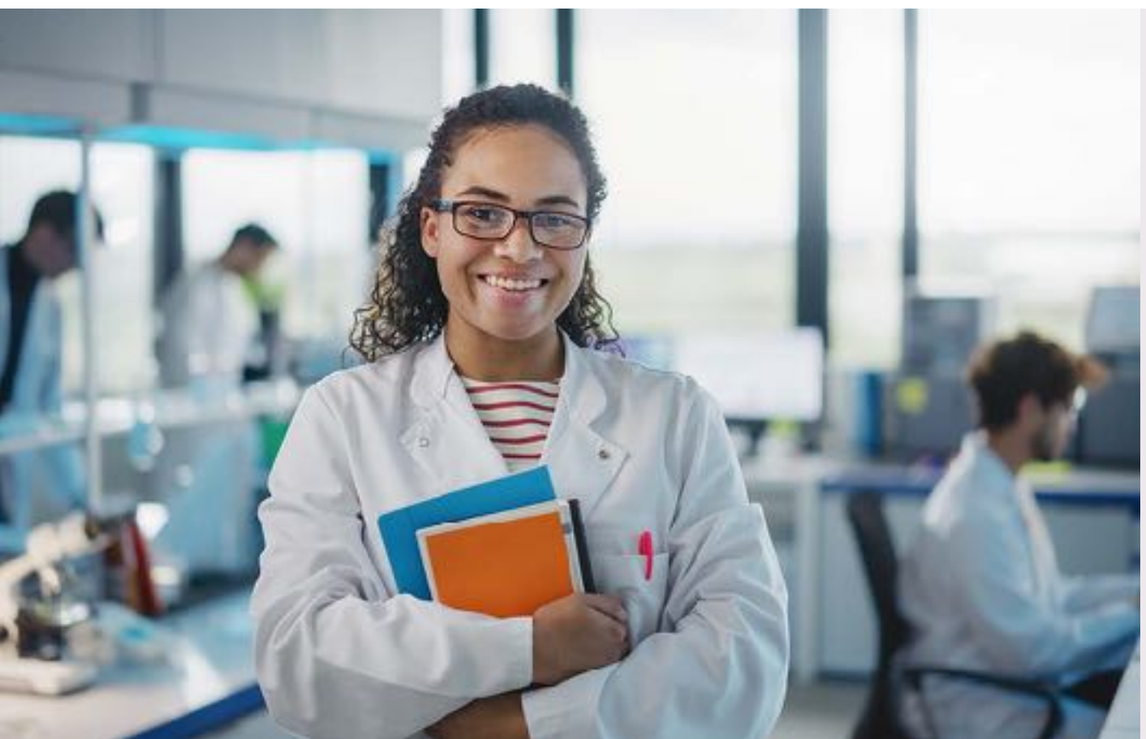
Buffer: Available in Tris 50mM, pH 7.5. Other buffers or customized formulation can be provided upon request.

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylaed lipids can be used upon request, as well as targeting molecules.

Storage/Stability: Store at +4°C for up to one week or several months at -80°C. Aliquot for storage. Do not freeze-thaw after aliquoting.

Use restrictions: For life science research use only.

Available sizes: 10µg, 20µg ,100 µg , 200 µg, 500 µg,bulk



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