



Synthelis®

Enzymes

Protein Catalog

Nox2, p22 Phox – Cytochrome b-245 light chain

PL039

Product specification

Acronym: p22 Phox (Nox2 subunit)

Synonyms: Synonyms

Origin species : Human

Protein reference : P13498 (UniProtKB)
AAA90925.1 (GenBank)

Family: Enzyme

Expression system: E.coli based CFPS

Format: Proteoliposomes

Protein sequence: Met1 – Val195

Tag : 6xHis tag (N-ter)

Cleavage site: Factor Xa

Product MW: 23,5 kDa

Application: Drug screening & discovery, antibody development, structural biology

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.

Recombinant protein sequence

His tag – factor X cleavage site –

MGQIEWAMWANEQALASGLLITGGIVATAGRFTQWYFGAYSIVAGVFVCLLEYPRGKRKKGSTMERWGQKHMTAVVKLFGPFT
RNYVRAVLHLLLSVPAGFLATILGTACLAIASGIYLLAAVRGEQWTPIEPKPRERPOIGGTIKQPPSNPPRPPAEARKKPSEEEA
AAAAGGPPGGPQVNPIPVTDVV



Quality analysis

Purity: > 60% (determined by Coomassie Blue stained SDS-PAGE)

Purification procedure: p22 Phox proteoliposomes are purified on a sucrose gradient. Further purification steps can be added if required.

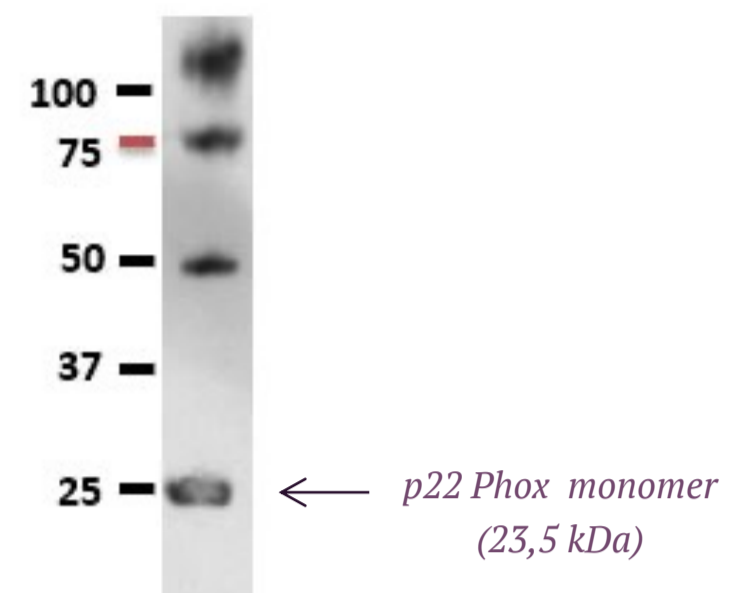


Fig.1 : Identification of p22 Phox in the proteoliposomes by Western Blot (using an anti-6xHis antibody).

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 Syntheliss' 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-dependent bactericidal activity of the macrophages. NBT is a dye with low reduction potential and performs intensively stained products—formazanes. NBT is easily phagocytized by cells and is reduced to formazane inside mitochondrium.

Results

The ability of the proteoliposomes to restore the in cellulo NADPH oxidase activity in the ROS-deficient macrophages has been analyzed. 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.

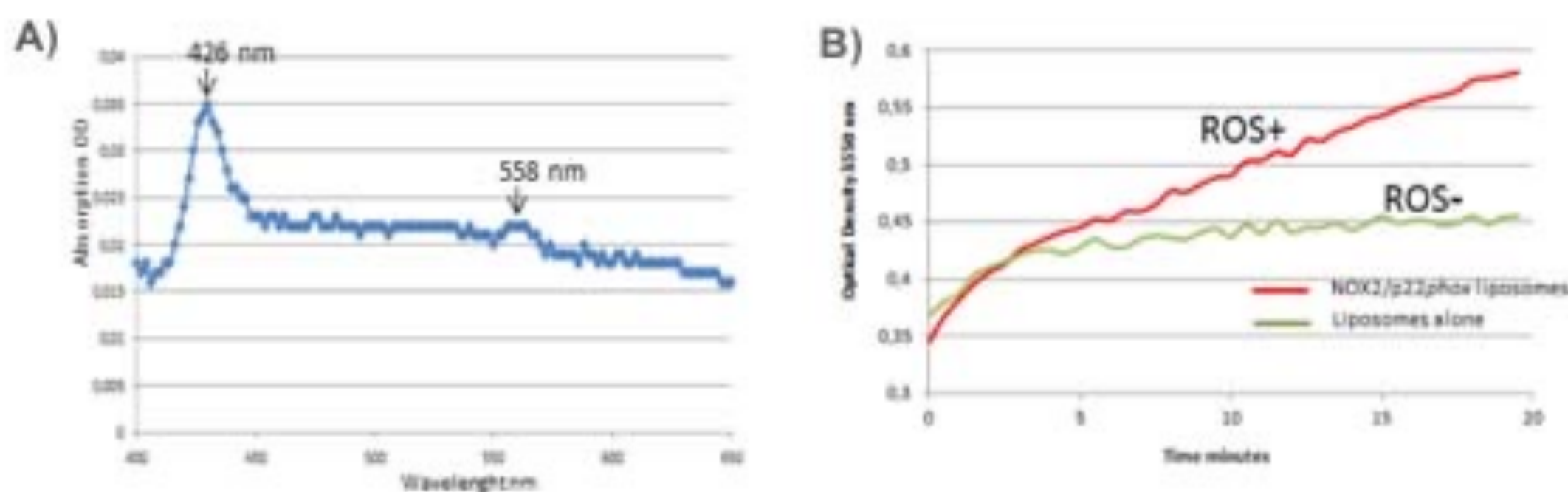


Fig.2: Analysis of Nox2/p22phox liposomes functionality. A) Differential spectrum of purified cytochrome b558 (Nox2/p22phox) in lipid vector. B) In vitro NADPH oxidase activity (in vitro assay).

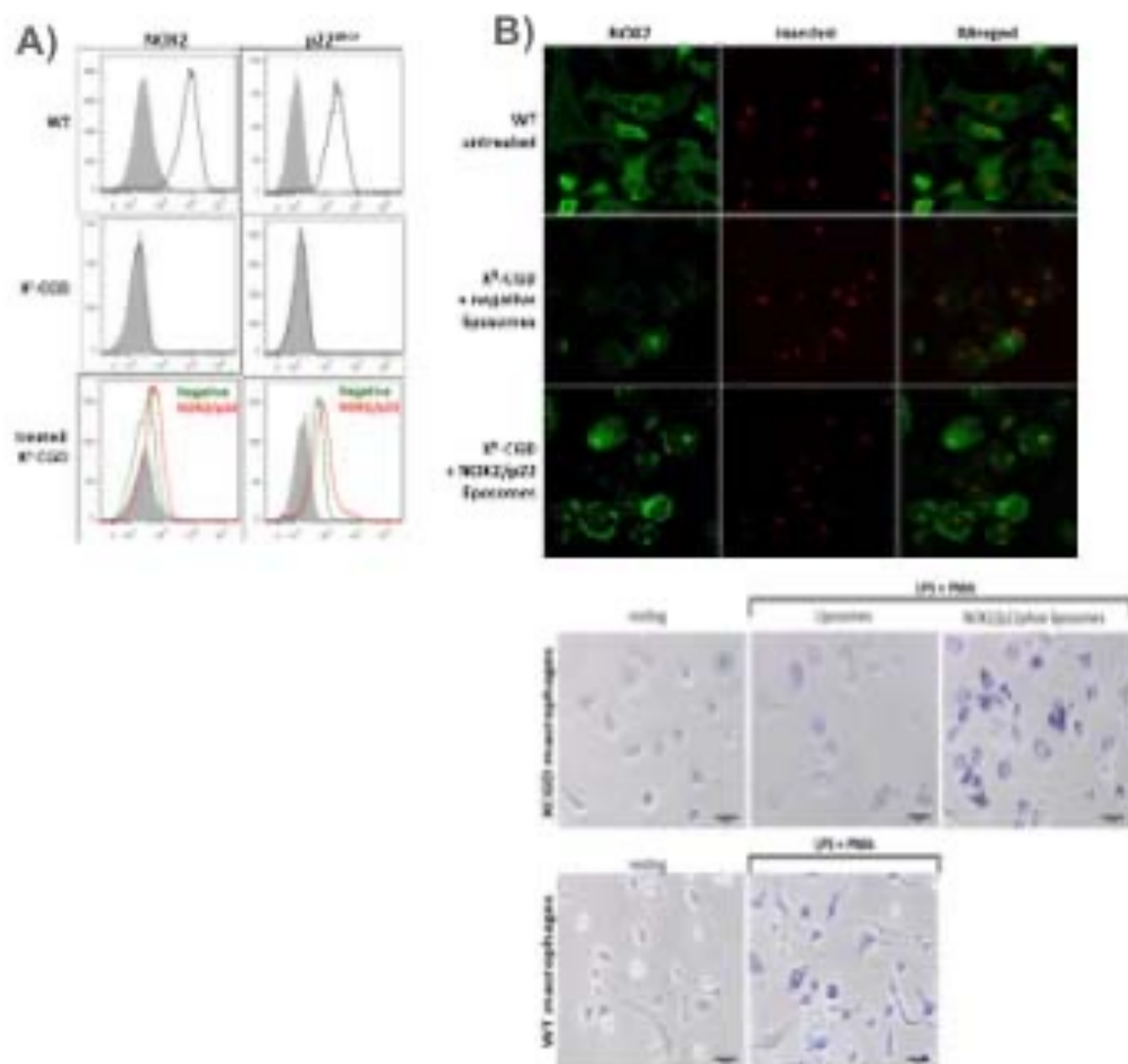


Fig.3: Analysis of in cellulo incorporation of NOX2/p22phox liposomes in the membranes of iPSCs-derived macrophages.

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.

For more detailed see: [Therapeutic effects of proteoliposomes on X-linked chronic granulomatous disease: proof of concept using macrophages differentiated from patient-specific induced pluripotent stem cells.](#) Julie Brault, Guillaume Vaganay, Aline Le Roy, Jean-Luc Lenormand, Sandra Cortès, Marie José Stasia in International Journal of Nanomedicine, 2017.

Formulation

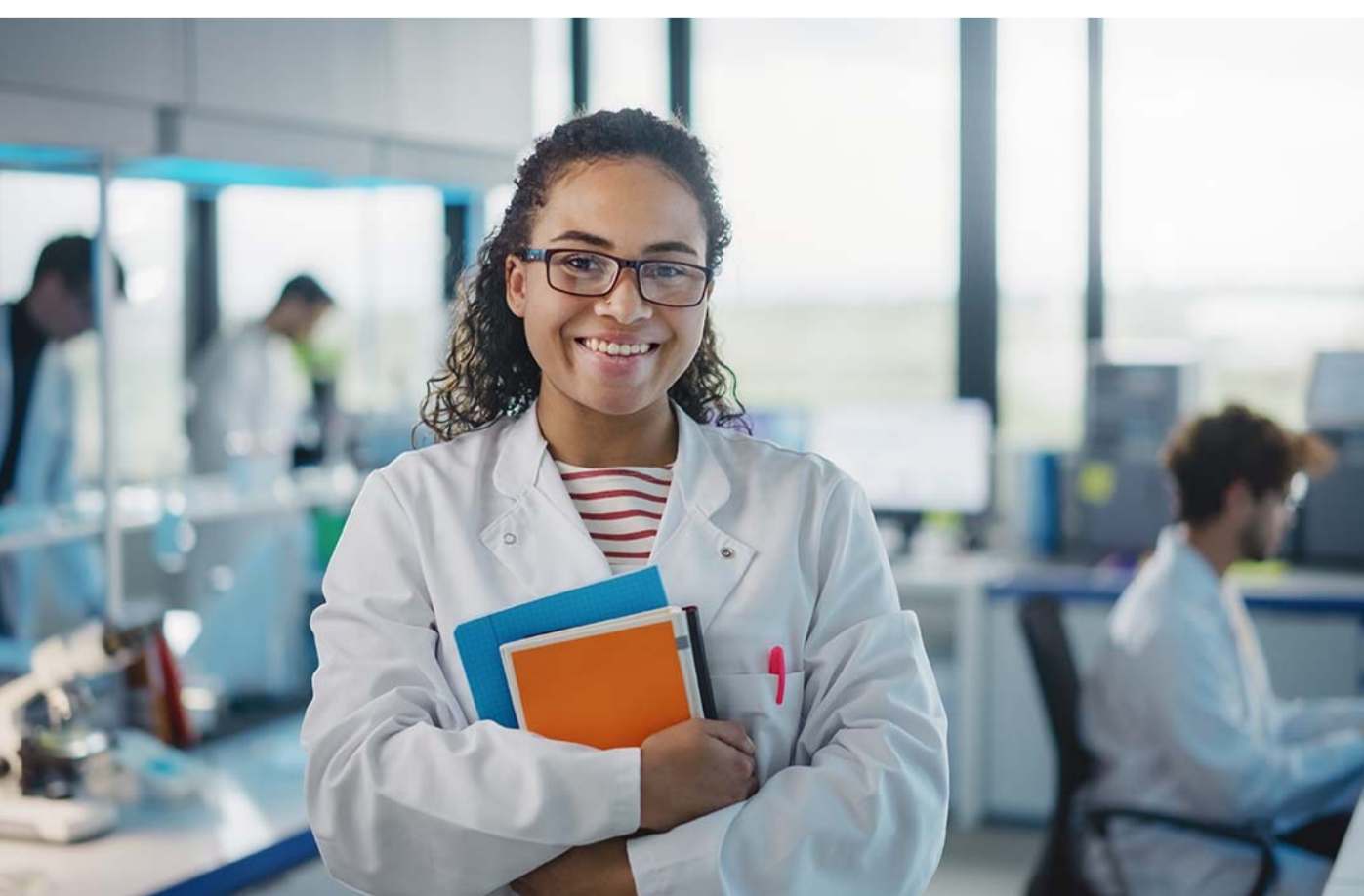
Buffer: Available in HEPES 50mM, pH 7.5 with cryoprotectants. Other buffers or customized formulation can be provided upon request.

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylated 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: 10 µg, 50 µg, 100 µg, customized quantity on request.



Need a specific amount, a quote or any additional information?
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