# Nox2, p22 Phox  – Cytochrome b-245 light chain

## Product specification

**Acronym:** p22 Phox (Nox2 subunit)
**Class:** Enzyme
**Origin:** Human
**Molecular weight:** 22 kDa
**Application:** Screening & display technologies, protein therapy.

**Purity:** >60%
**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:** p22 Phox  wild type protein

*Fig.1: AA sequence of p22 Phox protein*

```
        10         20         30         40         50
MGQICWAMWA NEQALASGLI LITGGIVATA GRITQWYTGA YSIVAGVTVC
        60         70         80         90        100
LLEYPRGKRK KGSTMERNGQ KYMIAVVKLF GPFTRNYYVR AVLHLLLSVP
       110        120        130        140        150
AGFIIATTIG TACIATAGGT YIIAAVRGFQ WTPIFPKPRF RPQTGGTTKQ
       160        170        180        190
PPSNPPPRPP ACARKKPSCC CAAVAAGGPP GGPQVNPIPV TDCVV
```

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

**Production conditions:** p22 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 > 60% as determined by SDS-Page and Coomassie Blue staining.

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

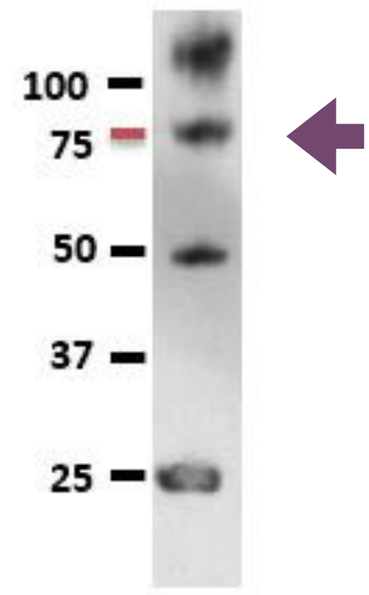



*Fig.2: Proteoliposome p22 Phox  after purification (Western blot identification).*

## 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-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.

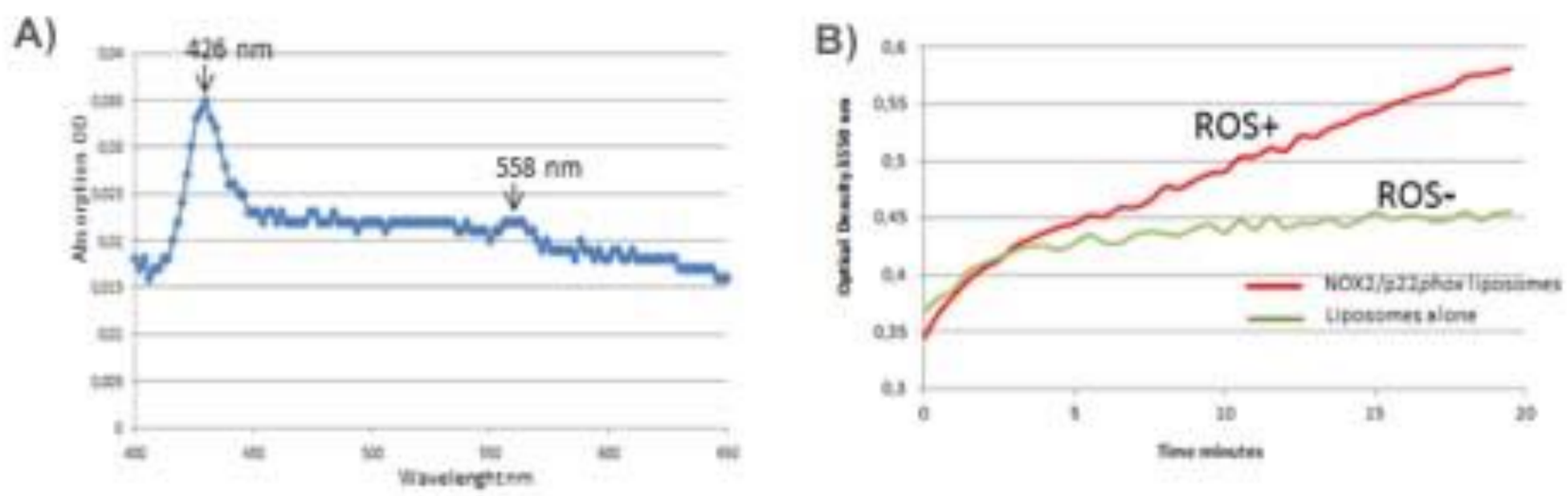



*Fig.3: Analysis of Nox2/p22phox liposomes functionality. A) Differential spectrum of purified cytochromeb558 (Nox2/p22phox) in lipid vector. B) In vitro NADPH oxidase activity (in vitro assay).*

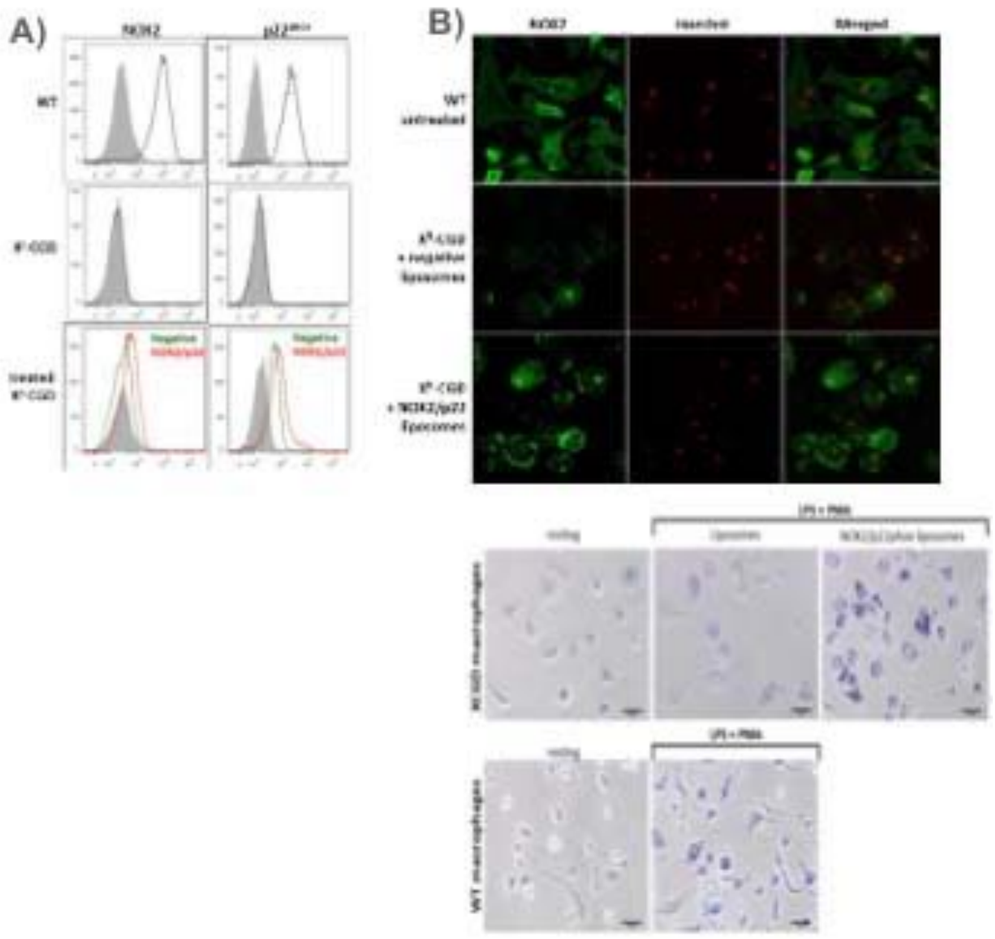



*Fig.4: Analysis of in cellulo incorporation of NOX2/p22phox liposomes in the membranes of iPSCs-derived macrophages.*

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

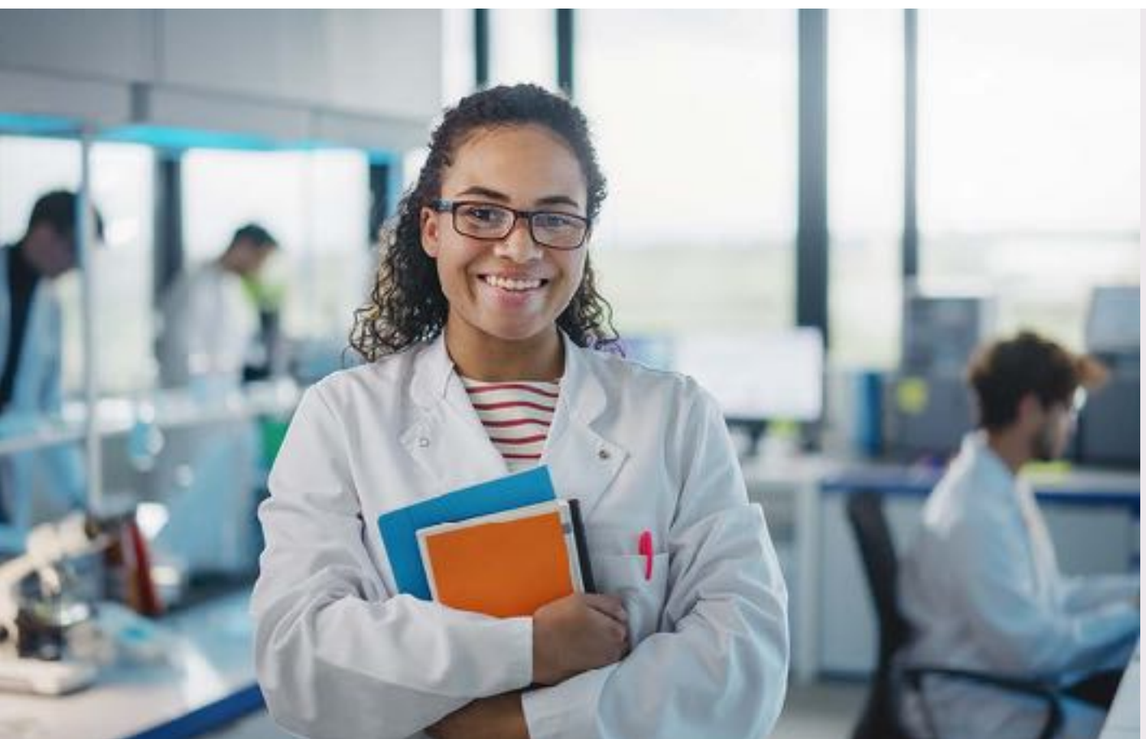
**Buffer:** Available in Tris 50mM, pH 7.5. Other buffers or customized formulation can be provided upon request.

**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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Synthelis

**T :** +33 (0)4 76 54 95 35
**E:** contact@synthelis.fr
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