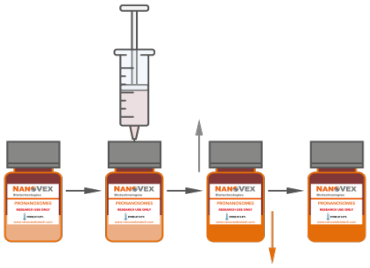
NANOVEX
Biotechnologies S.L.

Nanoencapsulation
PRACTICE KIT

Nanovesicles are closed bilayer structures able to entrap a wide range of compounds providing several advantages such as: encapsulated compound protection, increased bioavailability, controlled delivery, target delivery, great stability and masking undesired tastes, among others.

Pronanosomes are ready-to-use formulations to obtain nanovesicles which are able to encapsulate different compounds (Hydrophilic and lipophilic molecules, peptides, proteins,…) in a fast and simple way:



1. **Load**
2. **Shake**
3. **Nanovesicles are ready to use**

Size and distribution can be reduced by using vortex or homogenizer. Small Unilamellar Vesicles (SUV) with smaller sizes and narrower distributions are obtained after sonication of the product.

Applications

Medicine
- •Infectious diseases
- •Cancer therapy
- •Gene therapy
- •Vaccination
- •Parasitic diseases
- •Macrophage activation
- •Others

Food
- •Functional foods
- •Superfoods
- •Dairy products
- •Others

Others
- •Biotechnology
- •Diagnostics
- •Cosmetics
- •Bioengineering
- •New pharma products
- •Transfection

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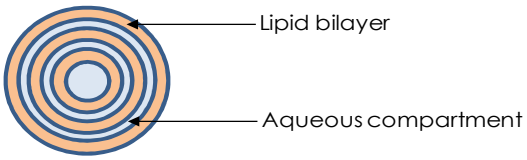
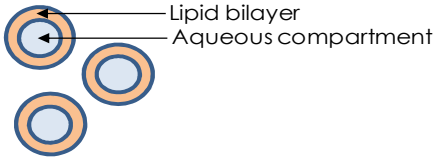
PRONANOSOME – HOW TO USE?



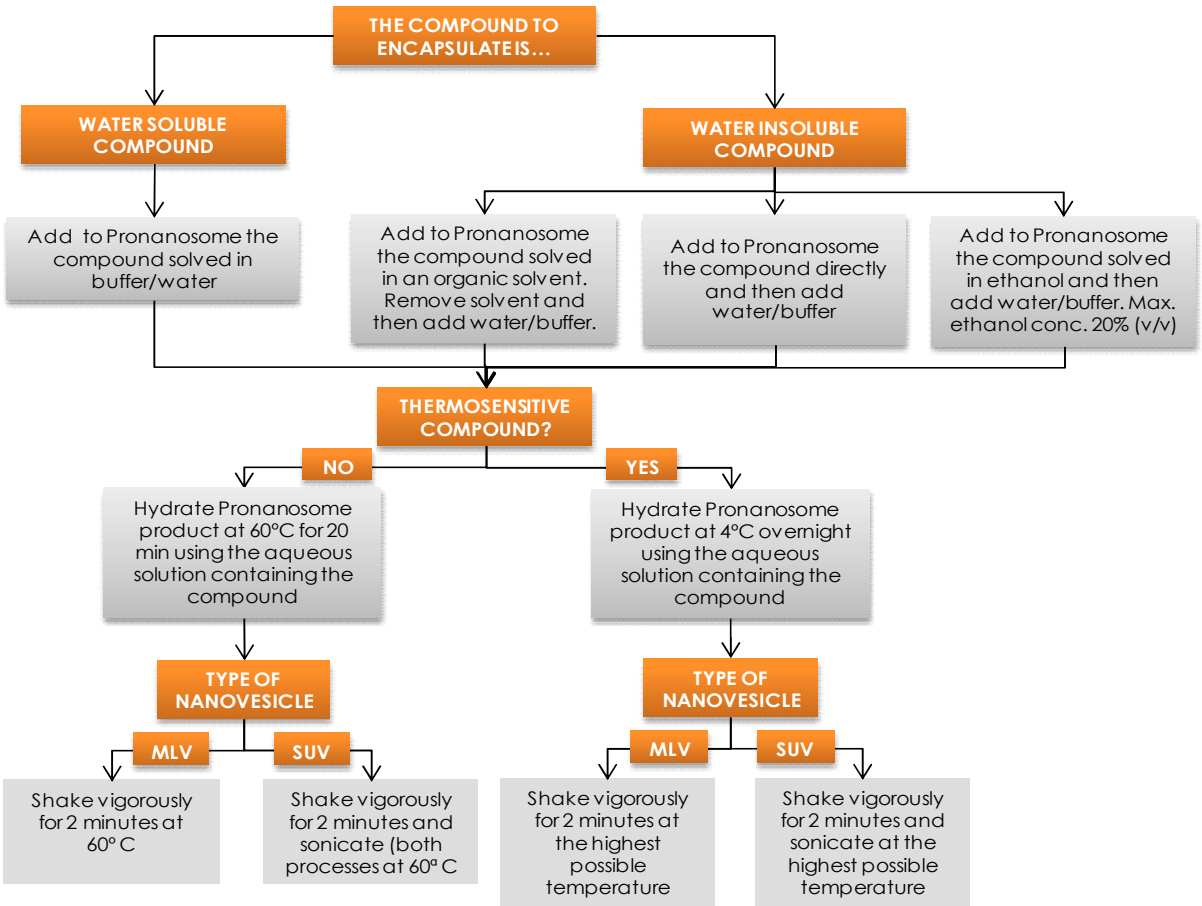
MultiLamellar Vesicles (MLV)

Characteristics
- High encapsulation efficiency
- Heterogeneous size
- Easy to obtain
- Cleared rapidly by the reticulo-endothelial system (RES)



Small Unilamellar Vesicles (SUV)

Characteristics
- High Lipid/Water ratio
- Relatively easy access to the cells of tissue
- Homogeneous size
- Low encapsulation efficiency in aqueous phase



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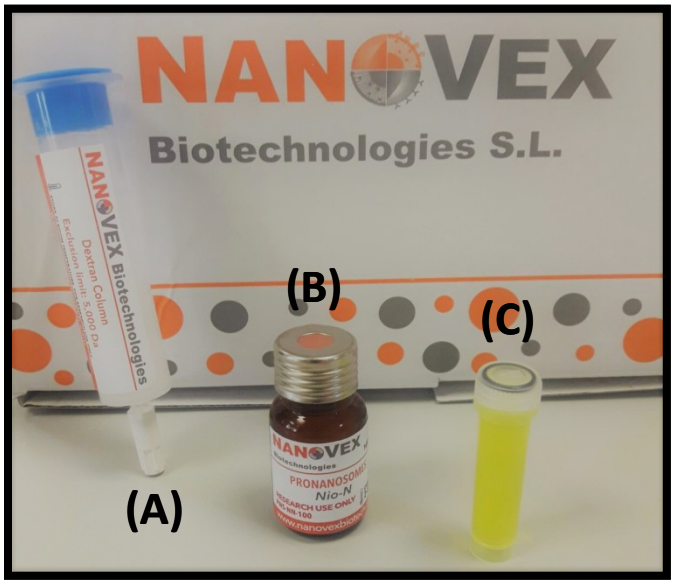

The kit is composed by

A. Size Exclusion Chromatography (SEC) Column
B. Pronanosome (to form nanovesicles)
C. Fluorescein solution100 µM.

NANOENCAPSULATION OF FLUORESCEIN IN NANOVESICLES

Remove the box from the refrigerator and allow it to reach room temperature before use.

1. Add the 2 ml of fluorescein (C) to the precursor (B).
2. Shake gently and immerse in a bath (bath temperature 55-65 ° C) for 20 minutes.
3. Shake up and down by hand vigorously for 2 minutes.
4. Sonicate in an ultrasonic bath (T ° bath 55-65 ° C) for 30 minutes.

NANOVESICLE PURIFICATION BY SIZE EXCLUSION CHROMATOGRAPHY.

First, it is necessary to prepare the column to subsequently carry out the separation of the NANOVESICLES from the non-encapsulated fluorescein

1. Shake the column to resuspend its contents. Then, place the column in a vertical position and allow the filling to settle again.
2. Carefully remove the storage solution from the top of the column.
3. Equilibrate the column by adding 50 ml of the desired working buffer.

NANOVESICLE PURIFICATION BY SIZE EXCLUSION CHROMATOGRAPHY (SEC)
Separation of nanovesicles from unencapsulated fluorescein

1. Equilibrate the SEC column with five column volumes (50 ml) of PBS.
2. Apply the reaction mixture (200 µl) to column. When the nanovesicles solution has entered into the column and the first fraction was collected, add PBS and continue collecting separate 1 ml fractions as they emerged from the column (Typically 30 fractions are collected).
3. For detection of nanovesicles and unencapsulated fluoresceín, 500 nm absorbance measurements in all fractions will be carried out (Typically fractions 4 to 6 will contain the nanovesicles, see the figure below).