# Sonoporation on exosomes

Technic: Permeabilize the membrane of exosomes

Goal: To encapsulate rhodamine in exosomes

Procedure:

1. Prepare a solution of 10 µL of exosomes, 2 µL of R110 and complete with PBS to 200 µL in a 1.5 mL microtube.
2. Pass the solution in the ultrasound bath for 3 minutes at 37°C.
3. On ice, sonicate the solution with the following protocol: 10% at a 5 seconds ON, 5 seconds OFF cycle for 30 seconds.
4. Let the tube incubate for 1 hour at 37°C.

Reagents and Solutions:

- Filtered PBS at 2 µm
- Exosomes at around $10^{12}$ particles/µL
- R110 at 5 mM

Equipment:

- Sterile microtubes
- Pipettes and sterile tips
- Ultrasound bath
- Sonication probe
- Dry bath incubator

Estimate time: ~30 minutes

Controls and Troubleshooting:
The amount of rhodamine can be changed as well as sonication factors.

# Electroporation on exosomes

Technic: Permeabilize the membrane of exosomes

Goal: To encapsulate rhodamine in exosomes

Procedure:

1. Prepare a solution of 10 µL of exosomes, 2 µL of R110 and complete with PBS to 150 µL in a microtube.
2. Transfer in an electroporation cuvette and electroporate.
3. Transfer back in a microtube then let incubate for 1 hour at 37°C.

Electroporation factors, two possible protocols:

1. Square waves:
   - Number of pulses: 10
   - Voltage: 3000 V
   - Pulse length: 5.0 ms
   - Pulse intervals: 5.0 s

2. Exponential decay
   - Number of pulses: 10
   - Voltage: 3000 V
   - Capacitance: 25 µF
   - Resistance: $\infty$ Ω

Reagents and Solutions:

- Filtered PBS at 2 µm
- Exosomes at around $10^{12}$ particles/µL
- R110 at 5 mM

Equipment :

- Sterile microtubes
- Pipettes and sterile tips
- Electroporator

- Electroporation cuvettes
- Dry bath incubator

Estimate time: ~30 minutes

Controls and Troubleshooting:
The amount of rhodamine can be changed, as well as electroporation factors.
All cuvettes had a 2 mm gap for the current to pass through.

## Purification with size exclusion chromatography (SEC)

Technic: Separation of particles by their sizes

Goal: To recover exosomes from non encapsulated particles (R100 or AuNps)

Procedure:

1. Rinse the column with 5 mL of distilled water, then 5 mL of PBS.
2. Pour the solution obtained from the encapsulation in the column.
3. When fully absorbed, directly pour PBS in the column so that it is never dry.
4. Recover the drops with microtubes in aliquots of 1 mL.
5. After 30 tubes ……..?
6. Resuspend and sample 30 µL of each tube in a 96 wells microplate.
7. Prepare a series of R100 or AuNps dilutions from $10^{-1}$ to $10^{-6}$ from the initial concentration used for the encapsulation and pour them in a separate row of the plate.
8. Read the plate in a fluorescence or absorbance reader depending on the agent encapsulated and plot the graph.

Reagents and Solutions:

- Distilled water
- Filtered PBS at 2 µm
- A solution of encapsulated exosomes (with R110 or AuNps)

Equipment:
- Sterile microtubes
- Pipettes and sterile tips
- SEC column
- Dark 96 wells microplate

- Fluorescence/absorbance reader

Estimate time: ~1 hour

Controls and Troubleshooting:
For fluorescence reading of R110, calibrate the machine on each of the dilution factors until obtaining the needed curve paying attention to the saturation.