

Date: \_\_\_\_\_/\_\_\_\_\_/\_\_\_\_\_

## PROTOCOL FOR MAKING VESICLES WITH MICROTUBULES INSIDE OF THEM

### Materials

In order to make vesicles with microtubules inside you will need the following:

- 2ml (can get them from Fisher Scientific) with Teflon caps. Also you will need 20ml vials with normal caps.
- Chloroform
- Gas tight syringes (I recommend the 100 $\mu$ l one). This can also be bought from Fisher Scientific.
- 100 $\mu$ l (at least) of DOPC and DOPS in chloroform at a concentration of 10 mg/ml (higher concentrations also work, but I have found the best results at this concentration). The initials DOPC and DOPS stand for Dioleoyl-Phosphatidylcholine and Dioleoyl-Phosphatidylserine. You can get them from Avanti Polar Lipids, Inc. in chloroform or as a powder. My personal experience is that asking for them in chloroform makes your life easier because you don't have to worry about concentrations. You will store these lipids in the freezer in aliquots of a few hundred of  $\mu$ l (100 $\mu$ l is recommended) using the 2ml vials.
- Teflon squares 1mm thick. You can get the sheet and cut it into ~1 cm side squares. Then you should scrape it with sand paper so that one of the surfaces is rough. After this , just sonicate for 30 min. and rinse very well. You can keep the squares in a Petri dish or in Methanol.
- TUBULIN (at concentrations higher than 2 mg/ml).

### Drying the lipid

-Take out from the freezer the lipids that are going to be used. Normally a mixture of DOPC and DOPS is used.

What concentration of lipid are you using? \_\_\_\_\_. The solvent is \_\_\_\_\_.

If it is a mixture, what are the proportions? \_\_\_\_\_.

-Take out a square of rough Teflon and place it on the bottom of a 20ml vial with the rough side facing up. Label the vial. What is the name of this vial? \_\_\_\_\_.

-Deposit the lipid solution on top of the Teflon square (be careful to not let the lipid flow down from the square because then it will almost all go to the glass bottom surface).

How much did you deposit? \_\_\_\_\_ $\mu$ l. ( cover all the surface of the square)

- Leave it on the bench with a loose cap for approximately an hour. It is ready when you are unable to observe any liquid on the surface.
- Place it in the dessicator under vacuum for at least four hours to remove any traces of the solvents. For how long did you leave it under vacuum? \_\_\_\_\_.
- Remove it and close the lid.

### **Pre-hydration and swelling of the vesicles**

-Uncover the vials (leaving the cap loose) and place them in a vapor saturated atmosphere for at least an hour. This will let some water get in between the layers of Phospholipid so that the swelling of unilamellar vesicles becomes easier. You can create this environment with a pipette tip box with pure water in the bottom at 60°C.

For how long did you leave them? \_\_\_\_\_.

-Remove the vial from the pre-hydration environment, and add 3-5ml of buffer that was previously filtered using a .22 $\mu$  filter. Close tightly the lid and place it back in the pre-hydration environment. This is not mandatory, but will prevent any evaporation if you didn't close the lid perfectly.

What buffer did you add? \_\_\_\_\_.

What was the osmolarity of this buffer? \_\_\_\_\_ mOsm.

-Let it there for approximately 4 hours. Vesicles will be formed within minutes, but in order to get a good amount of them; you need to let the lipid swell for at least 2 hours.

How much time did you let the vesicles swell?\_\_\_\_\_.

At this point, you should be able to see a white cloud floating on top of the Teflon square. This cloud is formed by millions of vesicles. The white-ish appearance is due to the scattering of light by these small vesicles and also to the polydispersity in size.

-Harvest the vesicles with a pipette (the small volume, 20 $\mu$ l, is recommended). You may obtain better results if you cut the pipette tip and always be very carefully to harvest without touching the Teflon.

-Place the harvested vesicles in a centrifuge tube.

-Label the tube. What is the name of the tube? \_\_\_\_\_.

Now you have a milky suspension of vesicles. To homogenize the suspension, mix it carefully by agitating it not very hard until the liquid has all the same white-ish color.

-Leave it on a tube rack on the bench. The vesicles will last for approximately a month.

### **Encapsulating Tubulin inside the Vesicles**

-Take the tubulin solution out of the Nitrogen dewar, and let it thaw on ice.

The concentration of the tubulin is? \_\_\_\_\_. How old is it? \_\_\_\_\_.

The buffer has the following formula: \_\_\_\_\_.

The osmolarity of the buffer is: \_\_\_\_\_.

-Place 20  $\mu\text{l}$  of your vesicle solution in the ice box.

-Label a 750  $\mu\text{l}$  centrifuge tube. This is the tube where you are going to prepare the final solution.

What is the name? \_\_\_\_\_.

-Deposit \_\_\_\_\_  $\mu\text{l}$  of cold lipid solution in it.

-Add GTP (or GMPCPP if you want to suppress dynamic instability) to the lipid solution so that the final solution has 1mM GTP.

How much did you add? \_\_\_\_\_  $\mu\text{l}$ .

What was the molarity of the original GTP solution? \_\_\_\_\_.

-Spin in the bench top centrifuge for less than 10 sec. And put the tube back in the ice box.

-Take \_\_\_\_\_  $\mu\text{l}$  of the tubulin solution and add it to the lipid-GTP solution.

What was the final concentration of tubulin? \_\_\_\_\_.

-Pull up and down once with the pipette. If you did not follow this step, what did you do? \_\_\_\_\_.

-Freeze this solution immediately in liquid  $\text{N}_2$  and let it sit on ice for 10-15 min. This is called the Freeze-Thaw technique, and it is a method for breaking the vesicles so that tubulin can get inside of them.

Diluting the vesicles and observing them under the microscope

For a better contrast in the imaging, it is recommended to dilute the vesicles into a salt solution. Salt has a slight lower index of refraction compared to that of the buffer, and this small change is enough to give a big contrast using the DIC technique in the microscope.

DESCRIBE YOUR RESULTS IN THE BLANK SPACE BELOW