

## Sample Preparation PRIOR TO Treatment with LPI™ FlowCell

### Membrane Preparation

There are many different ways to produce a membrane sample, and selection must be made according to each analysis objective. Described here is a general method, successfully employed for a wide number of applications of LPI™ FlowCell.

The first step is to lyse the cells in order to separate the soluble protein fraction from the membrane fraction. Depending on cell type, optimal methods should, ideally, be sought in the literature. Homogenizers using mechanical or liquid shear, sonication methods and osmotic lysis can successfully be applied. After lysis, nuclei and large fragments are then usually removed by low speed centrifugation, typically at ~400 x g for 5 minutes. After lysis and removal of nuclei, the sample is inspected under microscope to assure proper disruption of the cells and complete removal of nuclei, respectively.

Depending on the membrane type of interest (plasma membrane, specific organelles, whole cell membrane etc.), membrane material can then be collected from the post-nuclear supernatant by, for example, centrifugation methods. There are also purification kits targeting, for example, the plasma membrane available. To collect the main part of the whole cell membrane, >100,000 x g is typically required and ultracentrifuges are used. This is the preferred method and the collected membrane material can then be further washed and pelleted in buffer (usually supplemented with protease inhibitors) several times. (Nanoxis' applications rely on a Beckman Airfuge.)

### Vesiculation

Vesicles are typically produced either by extrusion or sonication. Tip sonication (sometimes referred to as probe sonication) is our preferred method. The tipsonicator use for most applications is a Vibra Cell (model 501) from Sonics & Materials Inc. The pelleted membrane fraction from the membrane preparation step is diluted with buffer (300 mM NaCl in 10 mM Tris, pH 8) to suitable concentrations (preferably 0,11 mg/ml total dry weight membrane material). Our protocol to create vesicles in the size interval 50150 nm in diameter includes setting the amplitude of the tipsonicator to 1020% and the total sonication time to 10 minutes. The pulse time and the resting time are normally both set to 5 s in order to reduce heating by the probe. To further reduce heating of the sample the sonication takes place in an icebath. Fragmentation is then confirmed by inspection in microscope.

# General membrane preparation protocol

