EV preparation protocol from prostate cancer cell lines

Two prostate cancer cell lines (PC3 and LNCaP) provided by the American Type Culture Collection (ATCC) were used as a model to produce prostate cancer-derived EVs that were distributed to all sites of the Cancer-ID project. Both cell lines were cultured at 37 °C and 5% CO_2 in RPMI-1640 with L-glutamine medium (Lonza, cat.# BE12-702F) supplemented with 10% v/v fetal bovine serum, 10 units/mL penicillin, and 10 µg/mL streptomycin. The initial cell density was 10,000 cells/cm^2 as recommended by the ATCC. Medium was refreshed every second day. When cells reached 80−90% confluence, they were washed three times with PBS and FBS-free RPMI medium supplemented with 1 unit/mL penicillin and 1 µg/mL streptomycin was added to the cells. After 48 h of cell culture, cell supernatant was collected and centrifuged at 1000g for 30 min. The invisible pellet containing dead or apoptotic cells and the biggest in size population of EVs was discarded. The supernatant was pooled, and aliquots of 50 µL were frozen in liquid nitrogen and stored at −80 °C. Size distribution and presence of the harvested EVs was assessed with nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM) images were taken to provide some examples of EVs.