Protocol for magnetizing cells in suspension with NanoShuttle using centrifugation:

1. Prepare cells* to a concentration of 2.5 x 10^6 cells/mL.
2. Add 250 µL NanoShuttle/mL of media (1 µL NanoShuttle/1 x 10^4 cells)

   Note: this amount can be further optimized, either by increasing or decreasing the amount of Nanoshuttle, depending on the targeted application.
3. Gently mix the cell and Nanoshuttle mixture using gentle pipette action
4. Centrifuge down at 100 g (or the speed generally used with the cell type being tested) for 5 min – **1st centrifugation**
5. Re-suspend cell pellet using gentle pipette action – at least 5x up and down or until pellet is well dispersed
6. Centrifuge down at 100 g (or the speed generally used with the cell type being tested) for 5 min – **2nd centrifugation**
7. Re-suspend cell pellet using gentle pipette action – at least 5x up and down or until pellet is well dispersed
8. Centrifuge down at 100 g (or the speed generally used with the cell type being tested) for 5 min – **3rd centrifugation**

Note: after each centrifugation step it should be clear that the pelleted cells acquire a more homogeneous brown color from the 1st (step 4.) to the 3rd (step 8.) centrifugation step.

9. Re-suspend cell pellet using gentle pipette action – at least 5x up and down or until pellet is well dispersed

Optional step for testing magnetization

a. Press a magnet to side of tube to attract magnetized cells
b. Collect supernatant

c. Centrifuge to see if there are or to determine amount of unmagnetized cells
d. If there are still a large amount of unmagnetized cells, this procedure can be repeated and we recommend optimization by increasing the Nanoshuttle amount in step 2.
   Why this further optimization step would be needed with particular cell types or cell culturing conditions?
   i. Cells can vary in size, therefore the overall surface area available to bind to the Nanosuttle will change.
   ii. Effective cell surface charge may vary depending on the media conditions, such as ionic content, ionic concentration, pH, presence of co-factors, and amount of serum.
   iii. The bullets i. and ii. rarely, or just slightly, influence Nanoshuttle labeling of cells, but it can be a factor.

* This protocol is generally recommended for cells from:
A. Suspension cultures
B. Cryopreserved cells to be thawed and cultured directly in 3D
C. Detached from adherent 2D monolayers.