Tissue Fixation for Immunohistochemistry:

The following outline is the protocol used by Nano3D Biosciences, Inc. to perform immunohistochemistry. Any standard fixation and immunohistochemistry protocols already used by your lab for the cell types and structures formed will also work with the **Bio-Assembler™** 3D structures. Notice however, that "Step 1" listed below should be included in your usual protocol to prevent the normal loss of cells during all pipetting procedures. Another important point is that depending on the antibody used, a different type of fixative might be needed, i.e. Methanol, Paraformaldehyde, Formalin, or a combination of fixatives.

Materials Needed:

Recommended Fixatives: 4% Paraformaldehyde (in PBS) or Formalin.

- 16% Paraformaldehyde (PFA) can be purchased from Electron Microscopy Sciences (#15710) and diluted to 4% in PBS.
- 10% Histological Formalin Solution can be purchase from Fisher Scientific (#SF98) and used directly.

10 mM Phosphate buffered saline (PBS)

- PBS tablets can be purchased from Sigma Aldrich (#P4417) and dissolved in deionized water.

4% Paraformaldehyde (PFA) or Formalin Fixation:

Step 1: Remove the magnet from the lid and place it under the bottom of the petri dish. The exposed side of the magnet should now be oriented up (as shown in Figure 1), so the cells will be magnetically directed to the bottom of the dish. This will hold your cells at the bottom of the dish and you will be able to do all the washes without worrying about losing the cells during the procedure.

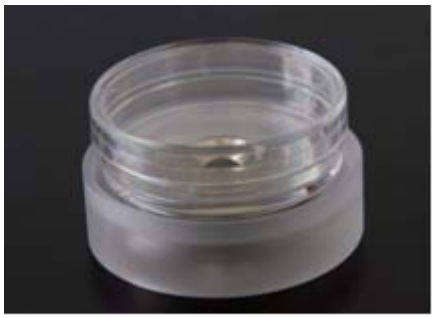

Figure 1. Magnet placed at the bottom of the petri-dish with the exposed side of the magnet oriented up.

Step 2: Gently pipette and discard media.

Step 3: Wash the cells 3 times with PBS.

Step 4: Add 1.5 ml of 4% PFA or 10% Formalin and incubate the cells for approximately 4 hours at room temperature. Note that the fixative and time of fixation may vary with the cell type, 3D structure, or target antigen. After fixation, the samples are ready to be embedded in paraffin and sectioned for the slides.

Step 5: Wash the cells 3 times with PBS.

Step 6: If desirable, transfer the cells (in PBS from step 4) into an Eppendorf tube (or other storage tubes) for storage at 4°C until paraffin embedding (see paraffin embedding procedure) is to be performed or direct staining of the 3D structures (see direct immunohistochemmistry without paraffin embedding).

Note: Some cell structures can be very sticky when using plastic pipettes, therefore, glass pipettes are better suited for transferring fixed 3D structures.