

Immunohistochemistry of Paraffin Embedded Tissue:

The following outline is the protocol used by Nano3D Biosciences, Inc. to perform immunohistochemistry of paraffin embedded tissue. Any standard fixation and immunohistochemistry protocols already used by your lab for the cell types and structures formed will work using **Bio-Assembler™** 3D structures.

Materials Needed:

Antigen retrieval solution: Dilute 10X solution to 1X

Permeabilization solution: 0.2% Triton X-100 in PBS

Donkey serum buffer: 10% donkey serum in PBS

Primary antibody solution: primary Ab (see manufacturer's directions for dilution) + 1% of 10% BSA, in PBS

Secondary antibody solution: secondary Ab (see manufacturer's directions for dilution) in PBS

Procedure:

1. Deparaffinize slides
 - a. 3x3 min in Xylene *Make sure paraffin is off at the end of this step*
 - b. In the meantime, heat water bath to 90°C.
2. Rehydrate sections
 - a. 1 min each in 100%, 95%, 80%, 70%, 50% ethanol
3. Rinse in running cold tap water **Note:** Use an upside down beaker to stand the staining jar on, tilt the rack, and aim water for one small corner.
4. Warm slides in dl for 3 min in water bath
5. Add slides to antigen retrieval solutions for 20 min
6. Wash in cold tap water for 10 min
7. Put in moisture chambers and create wells around sample with pap pen
8. Add 50-75 uL permeabilization solution per well for 15 min at RT *Only add enough to cover the well, do not overload*
9. Wash in PBS 3x5 min on shaker
10. Place slides back in moisture chamber.
11. On each well, add 50-100 uL donkey serum buffer in PBS for 1 h at RT
12. For experimental sections, remove serum and add primary antibody solution (see manufacturer's directions) for either 1 h at 37°C (in incubator) or overnight at 4°C (in fridge). *Controls have goat/donkey serum buffer left on. Longer incubation time allows for better antibody binding*
13. Wash in PBS 2x 5 min on shaker
14. Add secondary antibody solution (see manufacturer's directions) for 1 h at RT
15. Wash with PBS 2x 5 min on shaker
16. Add one drop (~25 µL) of VectaShield HardSet Mounting Medium *with DAPI* per section, and a small line of DAPI on the edge facing you.
17. Cover slip line side first
18. Leave overnight in the fridge. *(Make sure slides are open to air so it can dry. Do not leave in moisture chamber as it will not dry.)*