Quick Start Guide

nCounter® Vantage 3D™ DNA:RNA:Protein Solid Tumor Assay for FFPE

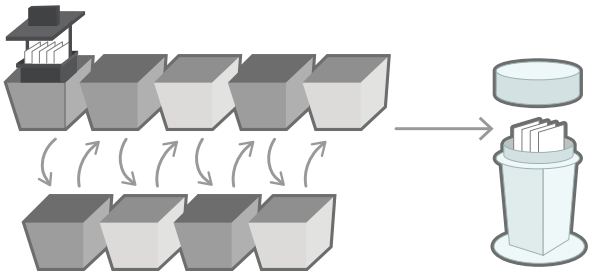
This quick start guide provides an overview of the protocol for preparation of DNA, RNA, and protein from FFPE samples. If you are a first-time user and for buffer preparation, please read the full protocols related to your Vantage 3D Assay and use this as a reference in subsequent experiments. Contact NanoString Support (support@nanostring.com) to receive additional assistance with this assay.

1 Extract RNA +/- DNA (See MAN-10050)

1. Purify RNA from FFPE tissue using Roche FFPET RNA Isolation Kit or Qiagen AllPrep DNA/RNA kit, if processing both DNA & RNA for Vantage 3D DNA SNV analysis
2. Measure and record RNA and DNA concentration
3. For SNV assay, amplify DNA according to MAN-10052 by creating a master mix of SNV Primer Pool, 5X dU Amp Master Mix, Nuclease-Free water. Add 8 µL master mix and 2 µL (5 ng) of DNA to each well before running the NanoString DNA Amplification Thermal Cycler Protocol.

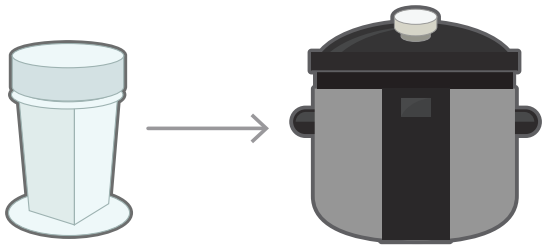
2 Deparaffinize and Rehydrate Slides (See MAN-10053)

1. Incubate slides in CitriSolv 3X for **5 minutes each**
2. Incubate slides in 100% ethanol 2X for **10 minutes each**
3. Incubate slides in 95% ethanol 2X for **10 minutes each**
4. Incubate slides in deionized H_2O 2X for **5 minutes each**
5. Transfer slides to a plastic Coplin jar containing 1X Citrate Buffer pH 6 and cover to prevent evaporation

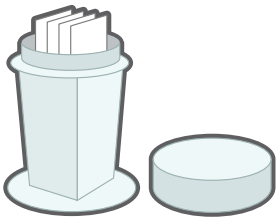


3 Epitope Retrieval (MAN-10053)

1. Detach the pressure cooker lid and make sure that the water in the pressure cooker is at the correct level per manufacturer's instructions
2. Place the Coplin jar with slides inside the pressure cooker

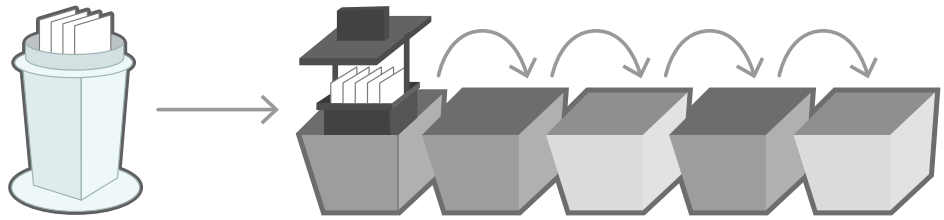


3. Attach the pressure cooker lid and run on high pressure for **15 minutes**
4. Release the pressure and carefully transfer the Coplin jar containing the slides to room temperature
5. Remove the lid of the Coplin jar and let stand for **25 minutes**

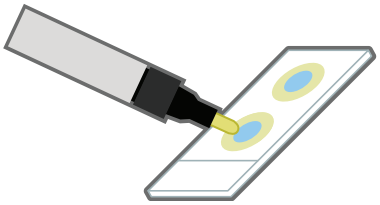


4 Primary Antibody Incubation (MAN-10053)

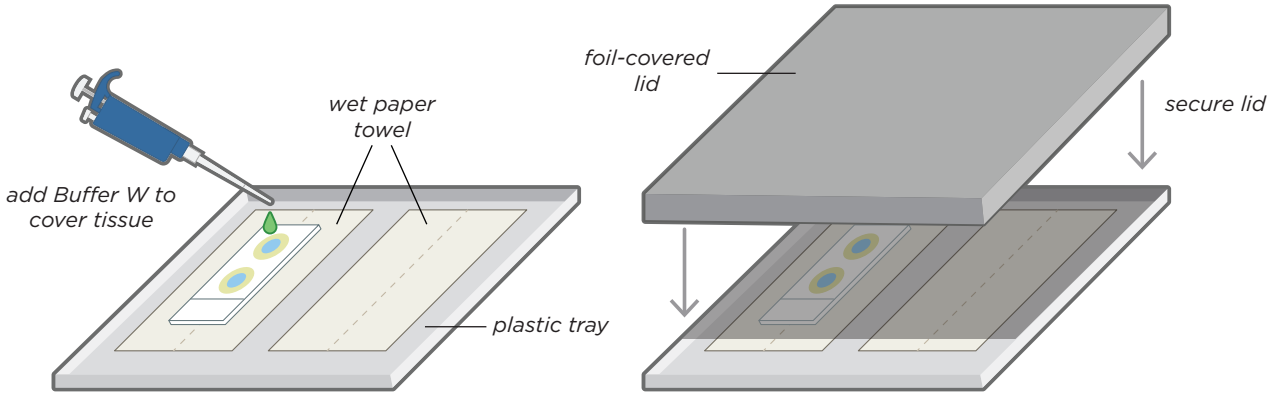
1. Wash the slides in 1X TBST 5X for **2 minutes each**



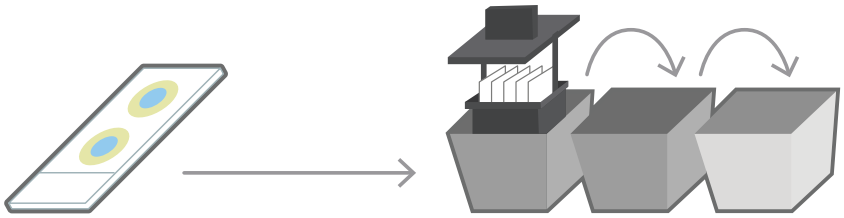
2. Carefully blot excess buffer from the edge of the slide with an absorbent wipe after the final wash
3. Draw a hydrophobic barrier around the tissue with a hydrophobic pen



4. Place the slide in a horizontal position and cover the tissue section with Buffer W
5. Incubate the slides in a closed humidity chamber (see below) for **1 hour** at room temperature



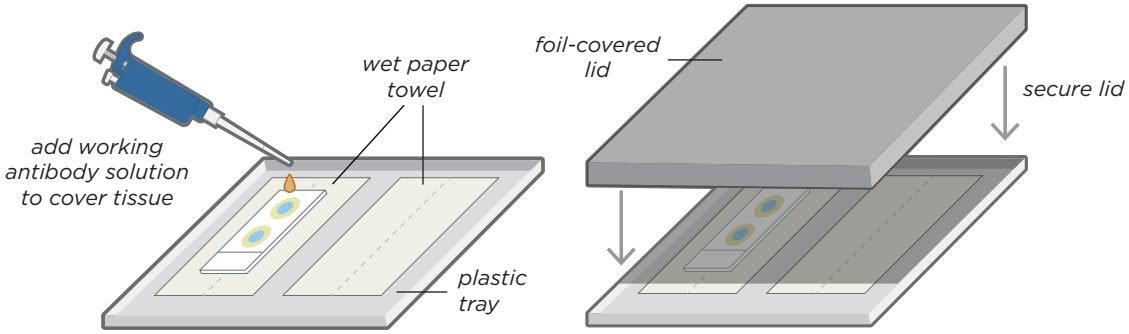
6. Carefully aspirate Buffer W
7. Transfer the slides to a staining rack and wash slides in 1X TBST 3X for **2 minutes** each



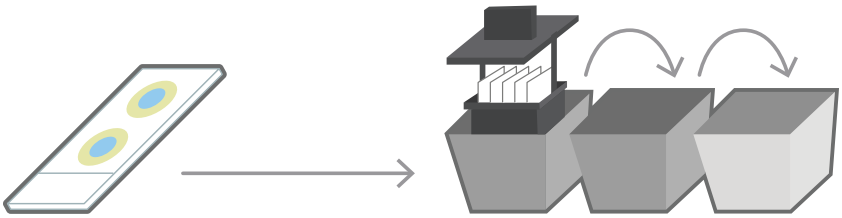
8. Make a working antibody solution by adding 64 µl of NanoString antibody mix to 2.5 mL of Buffer W
9. Carefully blot excess TBST from the edge of the slide with an absorbent wipe

4 Primary Antibody Incubation (MAN-10053) cont'd

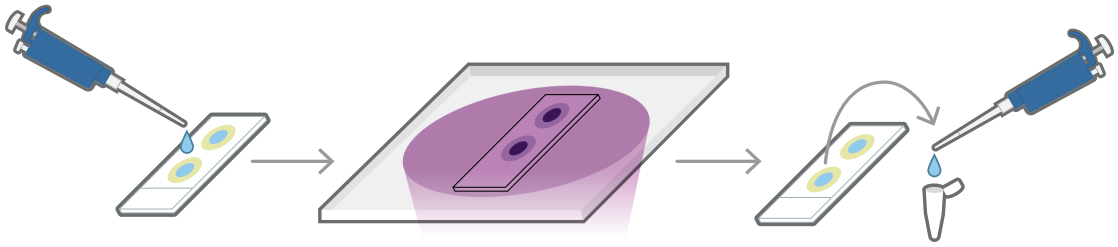
10. Place the slide in a horizontal position and cover the tissue section with the working antibody solution
11. Incubate the slides in a closed humidity chamber overnight at **4°C**. Minimize exposure to light



12. Aspirate working antibody solution
13. Transfer the slides to a staining rack and wash slides in 1X TBST 3-4X for **10 minutes** each



14. Carefully blot excess TBST from the edge of the slide with an absorbent wipe after the final wash
15. Place the slide in a horizontal position and cover the tissue section with Buffer T
16. Place slides directly onto a UV gel box and expose to UV for **3 minutes** to cleave tags from antibodies
17. Carefully mix the Buffer T containing cleaved oligo and transfer to a microcentrifuge tube



18. See appropriate manual for hybridization set up:
    MAN-10059 Protein Only
    MAN-10060 RNA:Protein (CodeSet)
    MAN-10065 RNA:Protein (TagSet)
    MAN-10055 DNA SNV

For more information, please visit 3d.nanostring.com

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