Quick Start Guide

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

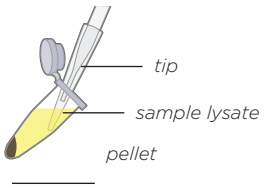
This quick start guide provides an overview of the protocol for preparation of DNA, RNA, and protein from cell or tissue lysate samples. If you are a first-time user and for buffer preparation, please read the full protocols related to your Vantage 3D Assay and use this as a reference in subsequent experiments.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

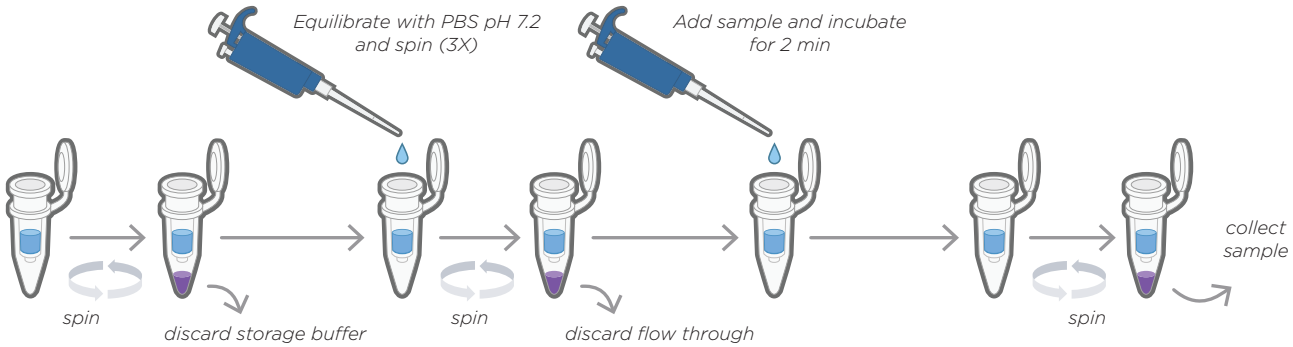
1 Prepare and amplify DNA (See MAN-10051)

1. Extract gDNA according to the methods described in MAN-10051 best suited for your sample.
2. Quantify DNA using a Qubit dsDNA HS Assay Kit and Qubit Fluorometer or a comparable PicoGreen fluorescence-based method
3. 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
4. Add 2 µL (5 ng) of DNA to each well before running the NanoString DNA Amplification Thermal Cycler Protocol.

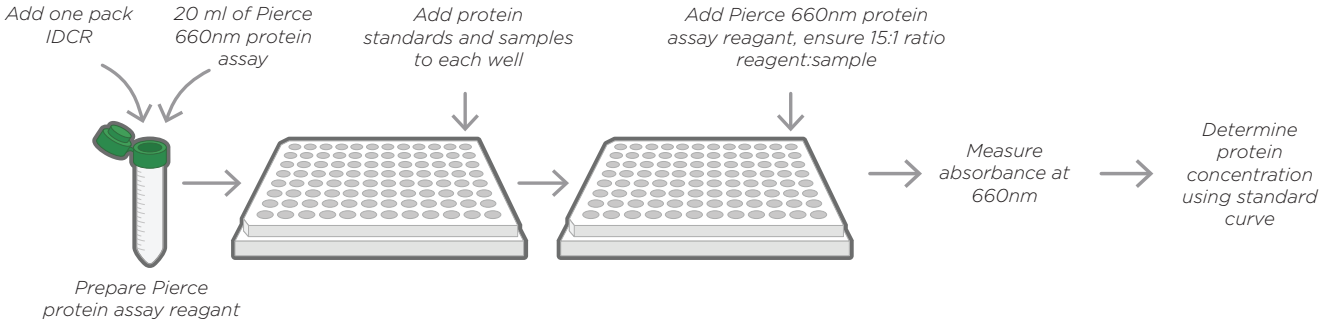
2 Prepare Sample Lysate (See MAN-10054)

1. Lyse sample by adding appropriate volume of SDS lysis buffer to achieve approximately 0.5 – 1.5 mg/mL protein concentration
2. Boil SDS lysate at **95°C** for **10 minutes**
3. Cool SDS lysate on ice, spin briefly, vortex, and spin briefly again
4. Remove detergent using the Pierce™ Detergent Removal Spin Columns





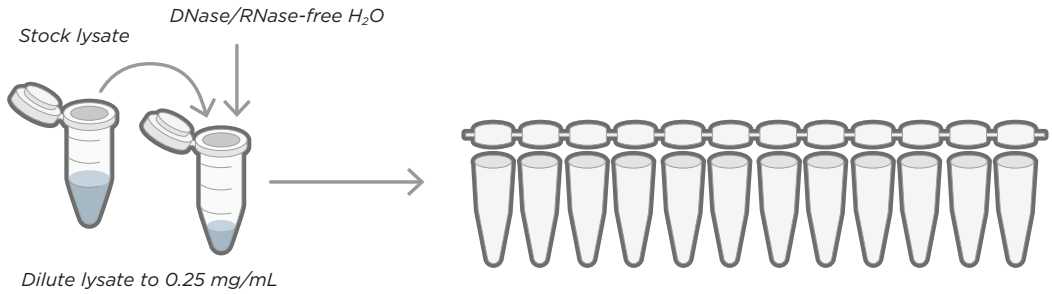
5. Determine protein concentration using the Pierce 660nm Protein Assay



6. After determining the protein concentration, samples can be stored at **-80°C** if desired

3 Prepare RNA Lysate (See MAN-10054)

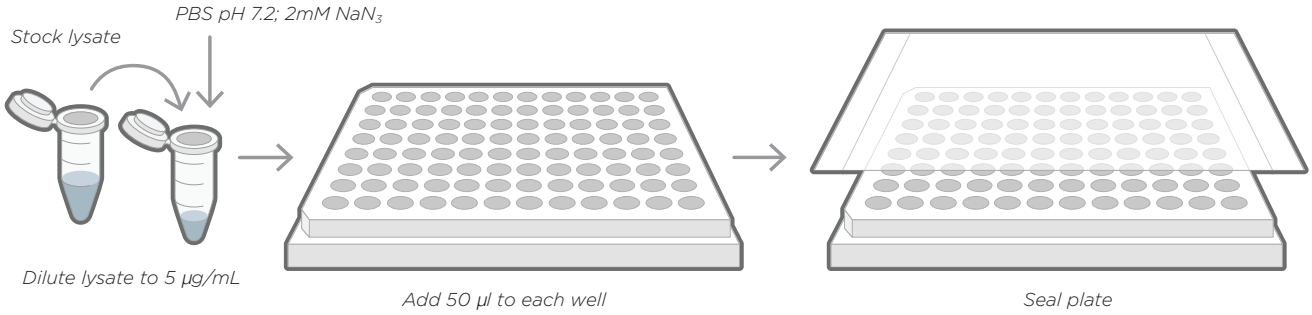
1. Dilute a small aliquot of the detergent-free lysate to 0.25 mg/mL using nuclease-free dH_2O. (A volume of 4 µl is required for hybridization)
2. Transfer diluted lysate to a 12-well strip tube



3. Store at **-80°C** until ready to proceed with hybridization

4 Bind Protein to Plate (See MAN-10054)

1. Dilute the detergent-free lysate to 5 µg/ml using PBS pH 7.2 with 2 mM NaN_3
2. Add 50 µl of the diluted lysate into a well of a protein-binding plate (MAXISORP plate) and seal with parafilm
3. Incubate for at least **2 hours** or **overnight** at room temperature

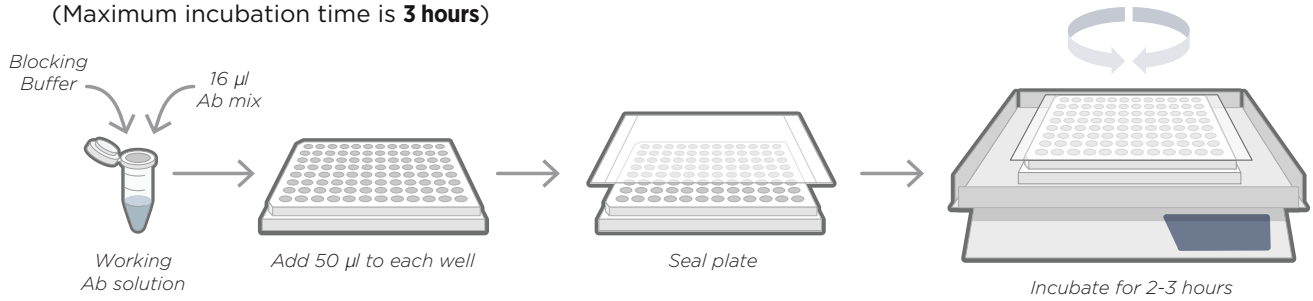


4. Add 200 µl of Blocking Buffer that has been pre-warmed to room temperature
5. Incubate for **5 minutes** at room temperature (Maximum incubation time is **1 hour**)
6. Remove and discard supernatant by flicking the plate into a sink and striking on a fresh paper towel hard enough to remove any residual liquid
7. Wash 3X by adding 250 µl of TBST pre-warmed to room temperature and incubating for **1 minute.** Remove and discard supernatant by flicking the plate into a sink and striking on a fresh paper towel hard enough to remove any residual liquid

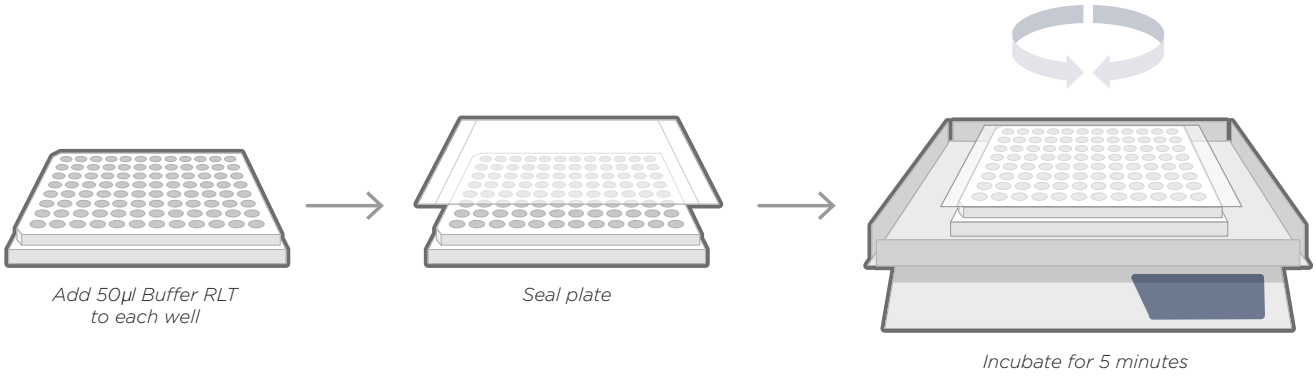
5 Prepare Protein Lysate (See MAN-10054)

1. Prepare a working antibody solution by adding 16 µl of the NanoString antibody mix to 625 µl of blocking buffer
2. Add 50 µl of the working antibody solution to each well
3. Seal the plate and incubate at room temperature for **2 hours** on an orbital shaker at 350-400 RPM

   (Maximum incubation time is **3 hours**)



4. Carefully remove and discard all supernatant with a single-channel pipette
5. Wash 6X by adding 250 µl of TBST pre-warmed to room temperature and incubating for **5 minutes.** Remove and discard supernatant by flicking the plate into a sink and striking on a fresh paper towel hard enough to remove any residual liquid
6. Add 50 µl of buffer RLT to each well
7. Seal the plate and incubate at room temperature for **5 minutes** on an orbital shaker at 350-400 RPM



8. Transfer the lysate to a 12-well strip tube

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

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

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