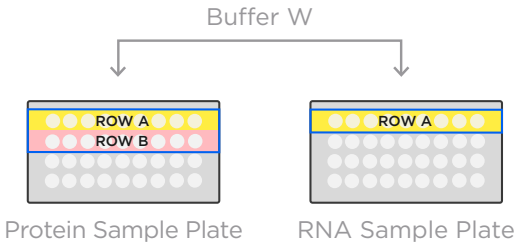
Quick Start Guide

nCounter® Vantage 3D™ RNA:Protein Immune Cell Profiling Panel for Cell Suspensions

with Universal Cell Capture Kit Cell Surface Compatible

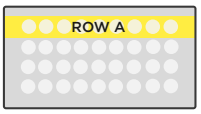
This quick start guide provides an overview of the RNA:Protein protocol described in MAN-10031. If you are a first-time user, please read the full protocol and use this as a reference in subsequent experiments. Contact NanoString Support (support@nanostring.com) to receive additional assistance with this assay.

1 Pre-block Protein & RNA Sample Plates (Section A)

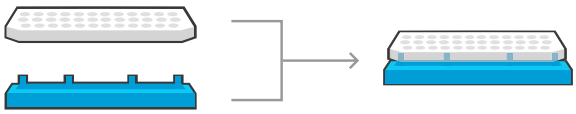


2 Bind Cells to Universal Cell Capture Beads (Sections B and C)

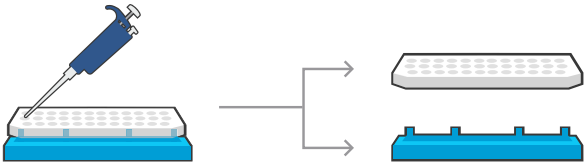
1. Collect a minimum of 20,000 cells (50,000 from primary cell samples)
2. Discard Buffer W from Protein Sample Plate
3. Add 200 µl of prepared cell solution to each well of Row A on Protein Sample Plate
4. Add 9 µl of Universal Cell Capture Beads to each well of Row A on Protein Sample Plate
5. Incubate plate for **30 minutes** at **4°C**



6. Place plate on magnetic separator
7. Leave plate on magnet for **5 minutes**



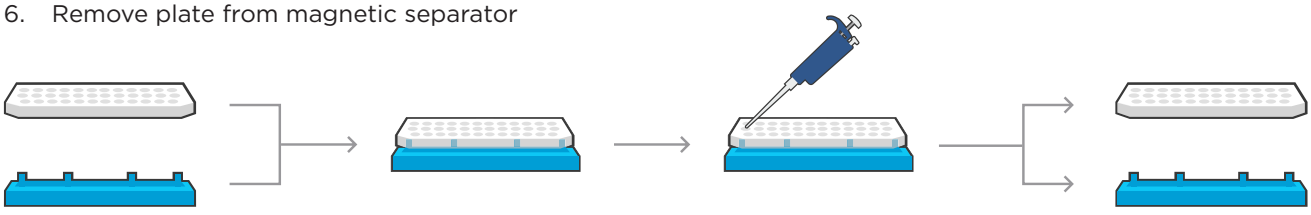
8. Remove supernatant, keeping the plate in contact with magnetic separator
9. Remove plate from magnetic separator



10. If necessary, resuspend beads in Fc receptor blocking solution, incubate for **10 minutes**
11. Add Buffer W to bring final volume to 200 µl

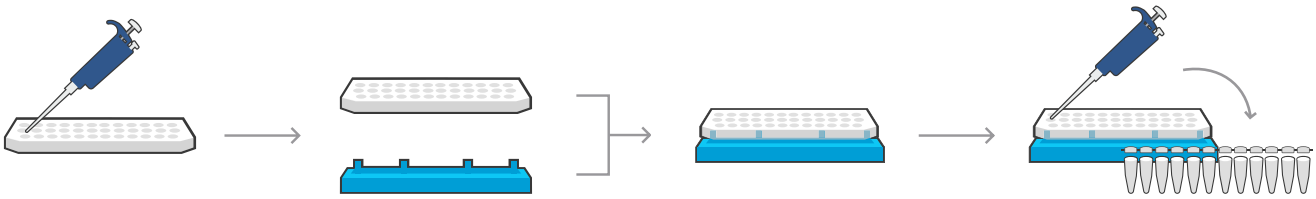
3 Prepare RNA Lysate (Section D)

1. Discard Buffer W from the RNA Sample Plate
2. Transfer 130 µl of each sample from the Protein Sample Plate to the corresponding well on the RNA Sample Plate
3. Place plate on magnetic separator
4. Leave plate on magnet for **5 minutes**
5. Remove supernatant, keeping the plate in contact with magnetic separator
6. Remove plate from magnetic separator



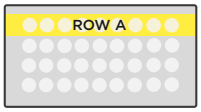
3 Prepare RNA Lysate (Section D), cont'd.

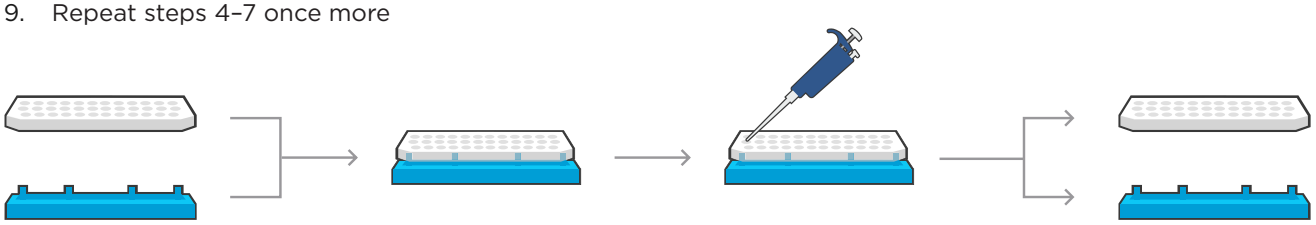
7. Resuspend beads in LH Buffer and pipette thoroughly to lyse cells on beads, incubate for **2–3 minutes**
8. Place plate on magnetic separator
9. Leave plate on magnet for **5 minutes**
10. Collect lysate, keeping the plate in contact with magnetic separator
11. Transfer lysate to a 12-well strip tube



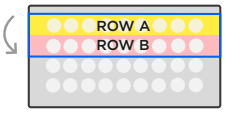
4 Prepare Protein Sample (Section E)

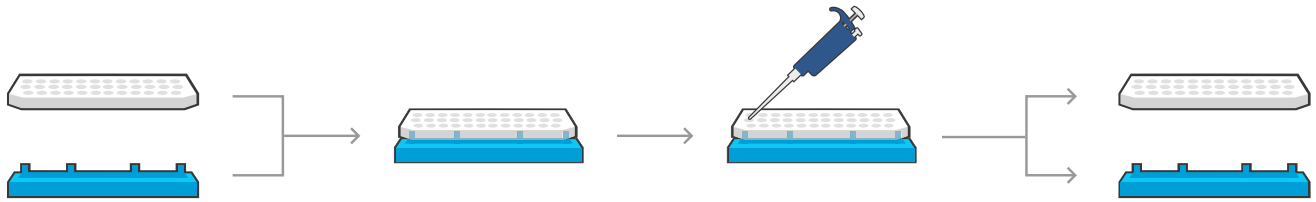
1. Add 130 µl of Buffer W to Row A of Protein Sample Plate
2. Add 10 µl of Ab Mix to Row A of Protein Sample Plate
3. Incubate for **30 minutes** at **4°C**
4. Place plate on magnetic separator
5. Leave plate on magnet for **5 minutes**
6. Remove supernatant, keeping the plate in contact with magnetic separator
7. Remove plate from magnetic separator
8. Gently resuspend beads in 200 µl Buffer W
9. Repeat steps 4–7 once more





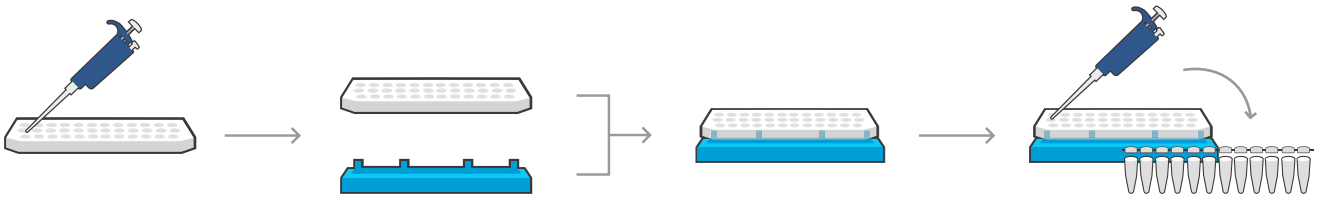
10. Gently resuspend beads in 200 µl Buffer W and transfer the samples from Row A to Row B of the Protein Sample Plate
    (Note that no additional transfers are made in subsequent washes)
11. Place plate on magnetic separator
12. Leave plate on magnet for **5 minutes**
13. Remove supernatant, keeping the plate in contact with magnetic separator
14. Remove plate from magnetic separator
15. Gently resuspend beads in 200 µl Buffer W
16. Repeat steps 11–14 once more





5 Prepare Protein Lysate (Section E)

1. Resuspend beads in LH Buffer and pipette thoroughly to lyse cells on beads, incubate for **2–3 minutes**
2. Place plate on magnetic separator
3. Leave plate on magnet for **5 minutes**
4. Collect lysate, keeping the plate in contact with magnetic separator
5. Transfer lysate to a 12-well strip tube



6. Denature protein lysates
7. Refer to MAN-10031 for RNA:Protein hybridization protocol

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