Quick Start Guide

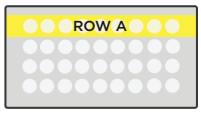
nCounter® Vantage 3D™ RNA:Protein Immune Cell Signaling Panel for Cell Suspensions

with Universal Cell Capture Kit Intracellular Compatible

This quick start guide provides an overview of the RNA:Protein protocol described in MAN-10032. 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 Bind Cells to Universal Cell Capture Beads (Sections B and C)

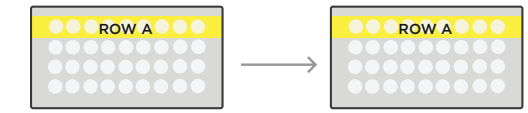
1. Collect a minimum of 20,000 cells (50,000 from primary cell samples) with a recommended collection of 50,000 cells (100,000 from primary cell samples)
2. Add 200 µl of prepared cell solution to each well of Row A on Protein Sample Plate
3. Add 9 µl of Universal Cell Capture Beads to each well of Row A on Protein Sample Plate
4. Incubate plate for **30 minutes** at room temperature (**RT**)



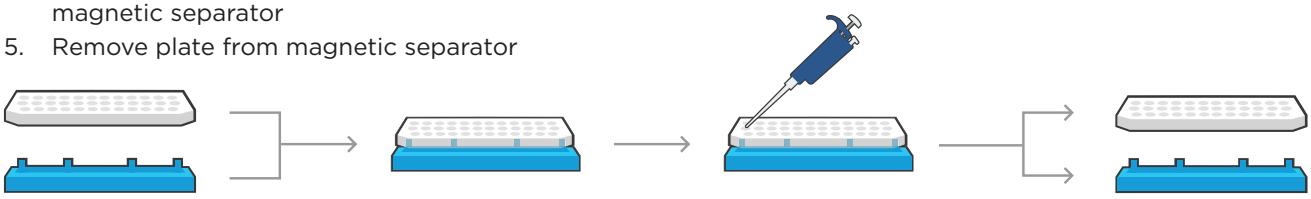
Protein Sample Plate

2 Prepare RNA Lysate (Section D)

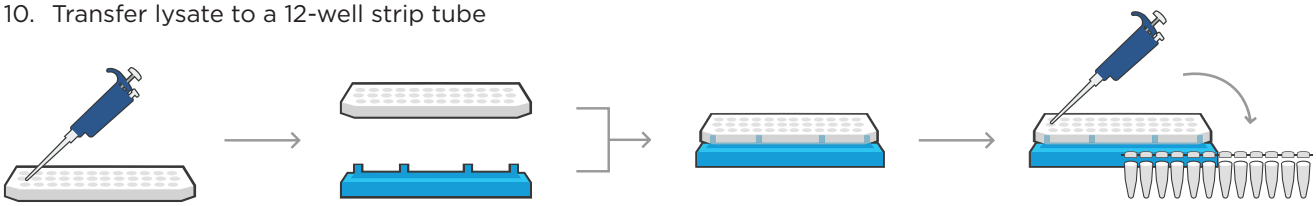
1. Transfer 130 µl of each sample from the Protein Sample Plate to the corresponding well on the RNA Sample Plate
2. Place plate on magnetic separator
3. Leave plate on magnet for **5 minutes**
4. Remove supernatant, keeping the plate in contact with magnetic separator
5. Remove plate from magnetic separator



Protein Sample Plate RNA Sample Plate

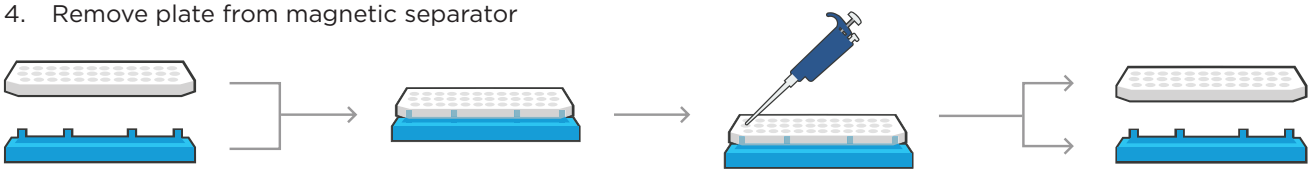


6. Resuspend beads in 200 µl of Buffer W
7. Repeat Step 2-5
6. Resuspend beads in Buffer LH and pipette thoroughly to lyse cells on beads, incubate for **2–3 minutes**
7. Place plate on magnetic separator
8. Leave plate on magnet for **5 minutes**
9. Collect lysate, keeping the plate in contact with magnetic separator
10. Transfer lysate to a 12-well strip tube

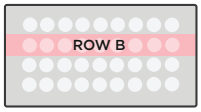


3 Prepare Protein Sample (Section E)

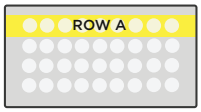
1. Immobilize the bead/cell complexes in the 70 µl of remaining sample by placing plate on magnetic separator
2. Leave plate on magnet for **5 minutes**
3. Remove supernatant, keeping the plate in contact with magnetic separator
4. Remove plate from magnetic separator



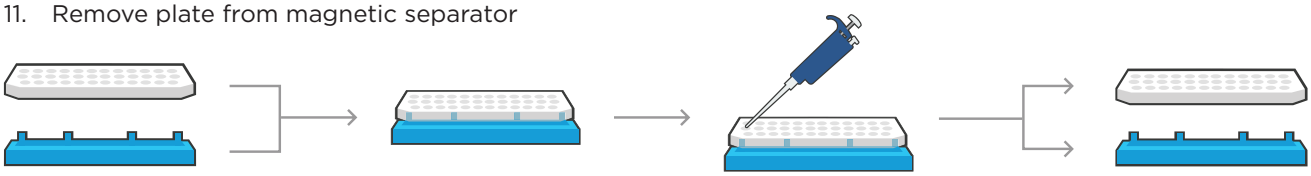
5. Add 300 µl 1X Buffer PW to Row B of the Protein Sample Plate



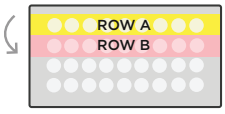
6. Fix cells by resuspending the beads in Row A in 200 µl of prepared 1X Fix Buffer



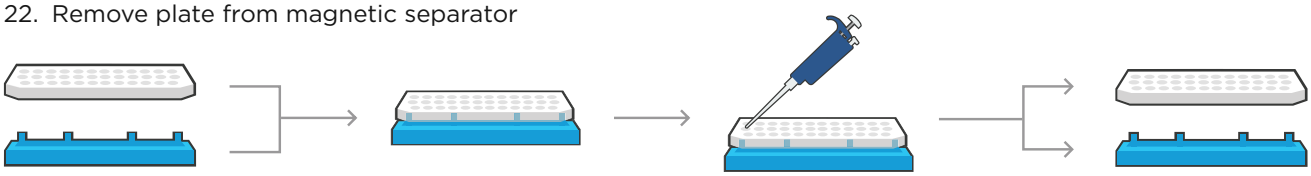
7. Incubate for **30 minutes**
8. Place plate on magnetic separator
9. Leave plate on magnet for **5 minutes**
10. Remove supernatant from Row A and B, keeping the plate in contact with magnetic separator
11. Remove plate from magnetic separator



12. Wash cells by resuspending the beads in Row A with 200 µl Buffer PW and transfer to Row B



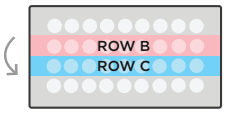
13. Repeat steps 8, 9, 11
14. If necessary, resuspend beads in 50 µl Fc receptor blocking solution, incubate for **10 minutes**
15. Add Buffer PW to bring final volume to 200 µl
16. Incubate for **30 minutes**
17. Add 10 µl of Ab Mix to Row B of Protein Sample Plate
18. Incubate for **60 minutes**
19. Place plate on magnetic separator
20. Leave plate on magnet for **5 minutes**
21. Remove supernatant, keeping the plate in contact with magnetic separator
22. Remove plate from magnetic separator



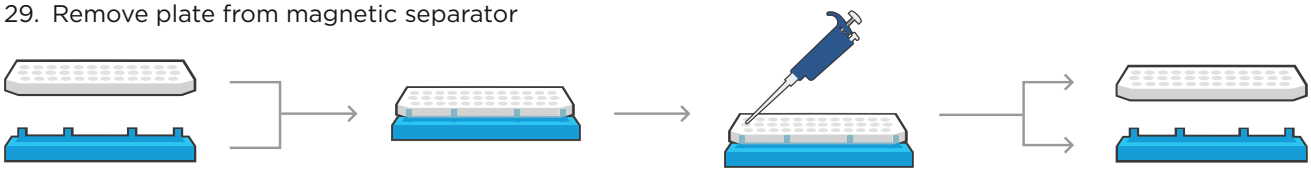
23. Gently resuspend beads in 200 µl Buffer PW
24. Repeat steps 19–23 twice more for a total of 2 washes with Buffer PW

3 Prepare Protein Sample (Section E), cont'd.

25. After the second wash, transfer the 200 µl Buffer PW plus sample/bead complexes from Row B to Row C of the Protein Sample Plate *(Note that no additional transfers are made in subsequent washes)*



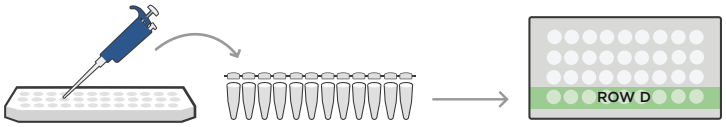
26. Place plate on magnetic separator
27. Leave plate on magnet for **5 minutes**
28. Remove supernatant, keeping the plate in contact with magnetic separator
29. Remove plate from magnetic separator



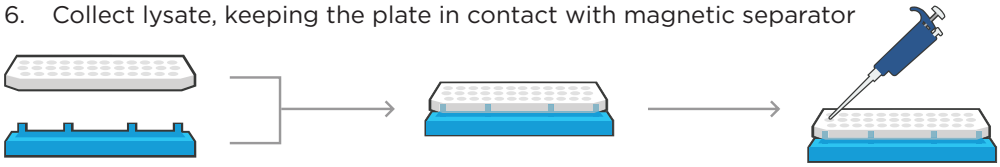
30. Gently resuspend beads in 200 µl Buffer PW
31. Repeat steps 26–29 once more
32. Carefully remove remaining residual buffer from each sample well after the final wash

4 Prepare Protein Lysate (Section E)

1. Resuspend beads in Buffer LH and pipette thoroughly to lyse cells on beads
2. Collect and transfer lysate to a 12-well strip tube
3. Incubate 12-well strip tube for **15 minutes** at **95°C** with a heated lid at **100°C**, then immediately ramp down to **4°C** or snap cool on ice for a minimum of **2 minutes**
4. Transfer cell lysates back to Row D of the Protein Sample Plate



5. Place plate on magnet for **5 minutes**
6. Collect lysate, keeping the plate in contact with magnetic separator



7. Refer to MAN-10032 for RNA:Protein hybridization protocol

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

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