

Ultra-Low Sample Inputs for RNA:Protein Profiling Experiments with nCounter® Vantage 3D™ Universal Cell Capture Kits

Introduction

Since its first commercial availability in 2008, the NanoString molecular barcoding system has been helping research scientists uncover greater insights from smaller samples. Direct, single-molecule detection of RNA was the first application on the nCounter® Analysis System, and the technology has since been expanded to other analyte types, including proteins in 2015.

The original nCounter® Vantage 3D™ RNA:Protein Immune Cell Profiling assay utilized centrifugation to collect cells during sample preparation steps and was compatible with as few as 150,000 cells (500,000 for peripheral blood mononuclear cells [PBMCs]), already lower than many other protein analytical techniques. In this tech note, we present a kit that reduces inputs 10-fold while retaining assay reproducibility. Part of the nCounter Vantage 3D portfolio of products, this kit contains cell capture probes covalently bound to magnetic beads. Now, with as few as 20,000 cultured cells or 50,000 PBMCs, researchers can profile up to 800 RNA and protein targets simultaneously.

10-Fold Input Reduction

To demonstrate the lower cell input requirements of the kit, we compared RNA:Protein results from the two protocols. Using the standard assay and centrifugation steps (spin method), we obtained data from 500,000 cells; using the kit and magnetic separation (Bead Isolation Method), we obtained data from 50,000 cells. We then plotted the signal outputs from these experiments and found a strong correlation ($R^2 > 0.96$) between the measurements of RNA and protein expression (FIGURE 1).

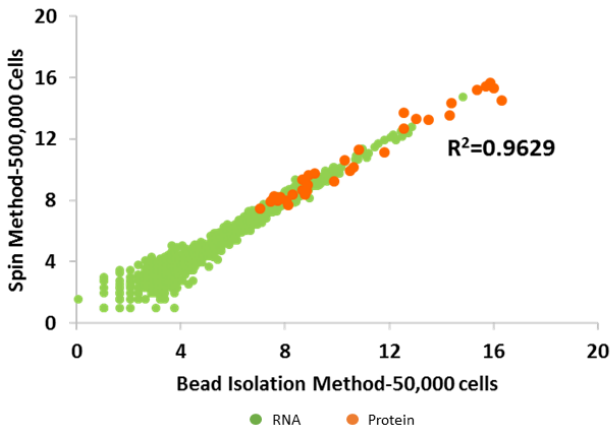


FIGURE 1 The bead isolation method, which utilizes the Universal Cell Capture kit, produces the same number of digital counts from RNA and protein targets as the spin/centrifugation method while using 10 times fewer cells.

Sample Handling and Appearance

The capture kit permits easier sample handling and visibility, which can help with tracking samples throughout the protocol. Without the beads, cells appear as semi-translucent pellets; following centrifugation, the beads are distinctly and intentionally brown (FIGURE 2).



FIGURE 2 Following magnetic separation, the cell capture kit beads appear as brown pellets in the bottom of the plate wells.

High Reproducibility

Although the capture kit uses fewer cells than the original method, the data are still highly reproducible. We compared results from two separate PBMC samples and found that even when the inputs vary by two-fold, the number of digital counts detected do not change significantly (FIGURE 3).

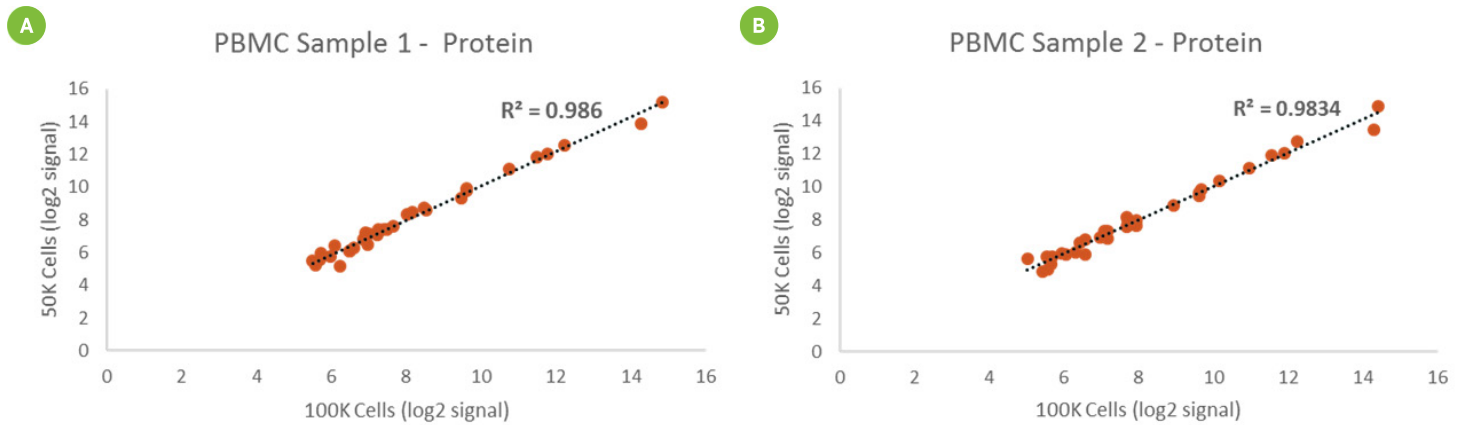


FIGURE 3 Using the bead method, two aliquots each from two different samples (A and B) result in very similar signals.

Summary

The nCounter Vantage 3D Universal Cell Capture Kits expand the applications of RNA:Protein Immune Assays to ultra-low inputs and allow researchers to learn more about samples that are otherwise inaccessible to standard analytical techniques.

NanoString Technologies, Inc.

530 Fairview Ave North
Seattle, Washington 98109 USA

www.nanostring.com

CONTACT US

info@nanostring.com
Tel: +1 888 358 6266
Fax: +1 206 378 6288

SALES CONTACTS

United States: us.sales@nanostring.com
EMEA: europe.sales@nanostring.com
Asia Pacific & Japan: apac.sales@nanostring.com
Other Regions: info@nanostring.com