
nCounter Vantage 3D Protein Barcoding Service (PBS)

Guidelines for Using Custom Barcoded Antibodies

Custom barcoded antibodies are supplied by NanoString's Vantage 3D Protein Barcoding Service at a concentration of 200 µg/mL and are supplied for use in NanoString assays. Up to three custom barcoded antibodies may be added as a spike-in to the nCounter Vantage 3D Protein Assays, in addition to the antibody mix supplied with the Vantage 3D Assay. Alternatively, custom barcoded antibodies can be combined and used as a custom antibody mix. These guidelines refer specifically to preparation of your antibody mix. Sample preparation should be performed as documented in your nCounter Vantage 3D manual with modifications only to the preparation and addition of antibody mix to your sample.

The custom barcoded antibody is supplied as a tester aliquot and master aliquot(s) in separate tubes. The tester aliquot may be used to optimize assay conditions for your specific custom barcoded antibody without repeated freeze-thawing of the master aliquot(s). The tester aliquot is provided as a 20 µL volume. Your master aliquot(s) should be further aliquoted and stored in volumes optimized for your desired NanoString assay. Note that there may be more than one vial of the master aliquot per antibody depending on overall yield after conjugation.

Preparing Custom Barcoded Antibodies

Use the following guidelines to dilute and then measure the performance of your custom barcoded antibody. These recommendations are provided as a starting point when determining the optimal concentration of antibody to be used in your desired NanoString assay. For cell suspension-based NanoString assays, a working range between 0.02 µg/mL–0.5 µg/mL final concentration of each antibody should be used, and the following guidelines result in a **0.1 µg/mL final concentration** for these assays. For lysate and FFPE-based NanoString assays, a working range between 0.05 µg/mL–2 µg/mL final concentration of each antibody should be used, and the following guidelines result in a **0.05 µg/mL final concentration**, which is the recommended starting point.

Note: Optimization of individual antibody concentrations beyond that described in Step 1 may be necessary.

Recommended Use for Cell Suspension-based NanoString Assays

Advanced Preparation

For one 12-sample cartridge, prepare 150 µL of a 2 µg/mL custom antibody mix as follows:

Perform a 1:100 dilution of the 200 µg/mL stock custom barcoded antibody by adding 1.5 µL per custom barcoded antibody to a final volume of 150 µL in 1X PBS (pH 7.4). Keep at 4°C until use. Use the dilute customer barcoded antibody mix within 48 hours.

Protein Sample Preparation

1. Modify the addition of antibody during protein sample preparation specific to your panel or sample/target type (see [MAN-10066, Cell Surface Protein Processing from Cell Suspensions](#)).
 - For spike-in applications, add 10 µL of the custom antibody mix prepared above in addition to the 10 µL of the antibody mix supplied with your Vantage 3D Assay to each well.
 - For fully custom protein analysis, add *only* 10 µL of the custom antibody mix prepared above.
2. Proceed with the Vantage 3D Assay protein sample preparation (see [MAN-10066, Cell Surface Protein Processing from Cell Suspensions](#)).

Recommended Use for Lysate-based NanoString Assays

Advanced Preparation

For one 12-sample cartridge, prepare 100 μL of 2 $\mu\text{g}/\text{mL}$ working solution as follows:

Perform a 1:100 dilution of the 200 $\mu\text{g}/\text{mL}$ stock custom antibody mix by adding 1 μL of each custom barcoded antibody to a final volume of 100 μL in Buffer WS; see [MAN-10054, Protein Processing for Lysate Samples](#) for details). Keep at 4°C until use.

Protein Sample Preparation

1. Modify the addition of antibody during protein sample preparation specific to your panel or sample/target type (see [MAN-10054, Protein Processing for Lysate Samples](#)). Total volume will be 640 μL .
 - For spike-in applications: add 16 μL of the custom antibody mix prepared above *and* 16 μL of the antibody mix supplied with your Vantage 3D Assay to 608 μL of Buffer WS.
 - For fully custom protein analysis: add *only* 16 μL of the custom antibody mix prepared above to 624 μL of Buffer WS.

Table 1. Protein Sample Preparation for Lysate-based Assays

	Custom Antibody Mix (μL)	Vantage 3D Assay Antibody Mix (μL)	Buffer WS (μL)	Total Volume (μL)
Spike-in	16	16	608	640
Fully custom	16	N/A	624	640

2. Add 50 μL of the working antibody solution to each well and proceed with the Vantage 3D Assay protein sample preparation (see [MAN-10054, Protein Processing for Lysate Samples](#)).

Note: All unused antibody should be discarded after dilution in Buffer WS.

Recommended Use for FFPE-based NanoString Assays

Advanced Preparation

For one 12-sample cartridge, prepare 154 μL of 5 $\mu\text{g}/\text{mL}$ working solution as follows:

Perform a 1:100 dilution of the 200 $\mu\text{g}/\text{mL}$ stock custom antibody mix by adding 1 μL of each custom barcoded antibody to a final volume of 100 μL in Buffer W; see [MAN-10053, Protein Processing for FFPE Samples](#) for details). Keep at 4°C until use.

Protein Sample Preparation

1. Modify the addition of antibody during protein sample preparation specific to your panel or sample/target type (see [MAN-10053, Protein Processing for FFPE Samples](#)). Total volume will be 2560 μL .
 - For spike-in applications, add 64 μL of the custom antibody mix prepared above *and* 64 μL of the antibody mix supplied with your Vantage 3D Assay to 2560 μL of Buffer W.
 - For fully custom protein analysis, add *only* 64 μL of the custom antibody mix prepared in Step 1 to 2560 μL of Buffer W.

Table 2. Protein Sample Preparation for FFPE-based Assays

	Custom Antibody Mix (μL)	Vantage 3D Assay Antibody Mix (μL)	Buffer W (μL)	Total Volume (μL)
Spike-in	64	64	2432	2560
Fully custom	64	N/A	2496	2560

2. Proceed with the Vantage 3D Assay protein sample preparation (i.e., antibody incubation according to [MAN-10053, Protein Processing for FFPE Samples](#)).

Note: All unused antibody should be discarded after dilution in Buffer W.

Data Analysis

All custom barcoded antibodies are accompanied by an add-in library file (ALF), which specifies the association between each custom barcoded antibody and its target. Information from the ALF must be merged with the reporter library file (RLF) from the original nCounter Vantage 3D Assay prior to scanning on the Digital Analyzer. Failure to merge an ALF with the original RLF will result in no count information being collected for targets of custom barcoded antibodies. Merging an ALF with the original nCounter Vantage 3D RLF will generate a new RLF that contains all probe information for both the custom barcoded antibodies and the original nCounter Vantage Assay. To merge an ALF or to convert an ALF into an RLF for custom protein only assays, please contact bioinformatics@nanosttring.com. A new RLF will be generated and e-mailed to you directly.

Once the optimal final concentration for your custom barcoded antibody has been determined, the master aliquot can be further aliquoted into volumes suitable for single use. Antibodies are pre-formulated with 10% rabbit serum and 2 mM sodium azide for long-term storage. Freeze aliquots at -80°C.

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