nanoString

CosMx™ Custom Protein Barcoding Service
Customize your CosMx Protein Assays

Expand your discovery further with high-plex CosMx™ Protein Assays with the CosMx Custom Protein Barcoding Service. Off-the-shelf CosMx Protein Assays currently enable up to 64-plex for protein imaging on CosMx Spatial Molecular Imager (SMI). Now researchers can use the Custom Protein Barcoding Service to barcode up to 8 antibodies of interest for use with CosMx Protein Assays
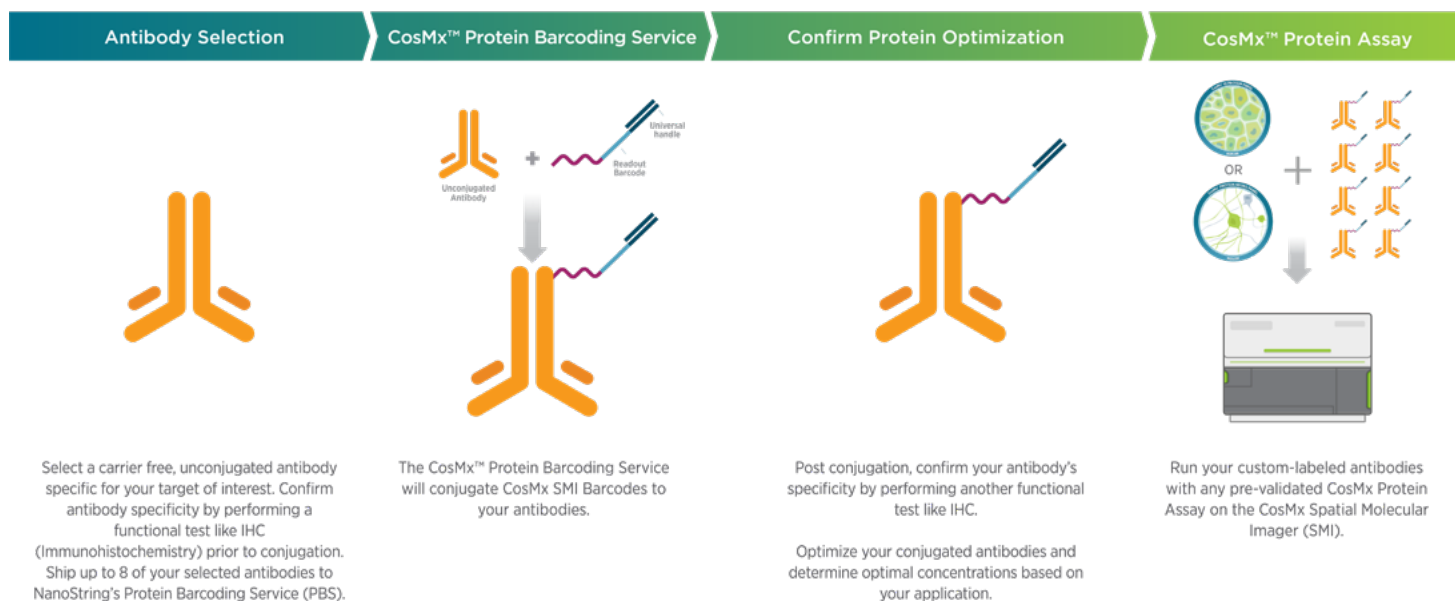

CosMx™ PROTEIN CUSTOM ADD-ON

Barcoding Service Submission Guidelines

Criteria	Requirement
Concentration & Volume	>250 µg at a concentration of >0.5mg/mL
Buffer	• 1x PBS with Sodium Azide at 0.02 –0.05% (Azide recommended but optional) • Buffer must be free of carrier proteins (e.g. BSA, gelatin) and cryoprotectants (e.g. glycerol)
Source	Mouse, Rat, or Rabbit

CosMx™ Protein Barcoding Service Process

See the table above for the recommended submission guidelines. For more detail, please see the CosMx PBS Antibody Optimization document. See the chart below for more information about how the Protein Barcoding Service works.

Antibody Selection	CosMx™ Protein Barcoding Service	Confirm Protein Optimization	CosMx™ Protein Assay
Select a carrier free, unconjugated antibody specific for your target of interest. Confirm antibody specificity by performing a functional test like IHC (Immunohistochemistry) prior to conjugation. Ship up to 8 of your selected antibodies to NanoString's Protein Barcoding Service (PBS).	The CosMx™ Protein Barcoding Service will conjugate CosMx SMI Barcodes to your antibodies.	Post conjugation, confirm your antibody's specificity by performing another functional test like IHC. Optimize your conjugated antibodies and determine optimal concentrations based on your application.	Run your custom-labeled antibodies with any pre-validated CosMx Protein Assay on the CosMx Spatial Molecular Imager (SMI).

Unconjugated Antibody Selection and Validation

Make sure to choose targets that are abundantly expressed to achieve a strong and specific signal. In addition, choose an antibody that has been recommended or has been validated for immunofluorescent (IF)/ immunohistochemistry (IHC) assays by the antibody provider, preferably in the same antigen retrieval buffer as used in CosMx Protein assays(citrate buffer, pH 6.0).

Antibodies must be provided in carrier-free buffer and free of BSA, gelatin or cell culture supernatant. While antibodies in glycerol containing buffers can still be used for conjugation, overall conjugation efficiency may be affected. Addition of sodium azide is recommended to avoid potential contamination during storage.

Pre-Validation Step Before Conjugation

Before protein barcoding, perform a standard IHC staining to ensure strong and specific antibody binding. Consult the Human Protein Atlas (proteinatlas.org), Allen Brain Map for both human and mouse, or other databases or publications for guidance.

Post-Conjugation

Conjugated (barcoded) antibodies are provided at a concentration of 200 µg/mL in a buffer of PBS and sodium azide and come with 16 slides worth of imaging reporters. Additional reporters are also available for purchase. A test aliquot is also provided for post-conjugation testing. Antibodies should be stored at 4°C for use within two weeks or aliquoted and kept at -80°C for long term storage. Avoid repetitive freeze thaw cycles during storage.
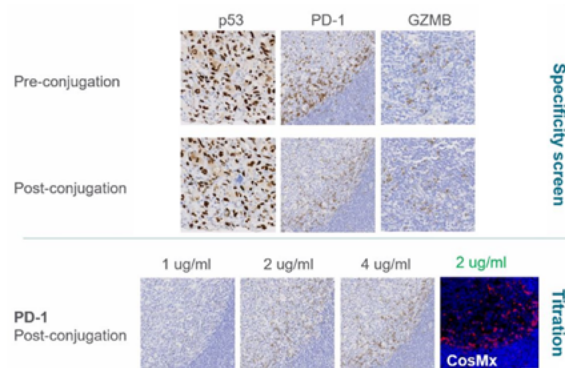

Figure 2: Pre– and Post– Conjugation antibody screen

Ordering Information

The Custom Protein Barcoding Service typically takes between 4-8 weeks from NanoString receipt of antibodies.

Product	Catalog Number
CosMx™ Protein Custom Add-On	CMX-PBS-1AB
[Optional] CosMx™ Custom Protein Reporter Kit 1	CMX-Custom-Reporters-1
[Optional] CosMx™ Custom Protein Reporter Kit 2	CMX-Custom-Reporters-2

Selected Panel References

• Protein Barcoding Service Webpage (including the Antibody Submission Form and Custom Antibody Optimization guidelines): nanostring.com/cosmx-pbs/

• CosMx SMI Manual Slide Preparation User Manual: university.nanostring.com/cosmx-smi-manual-slide-preparation-user-manual

To learn more about the Custom Protein Barcoding Service and download the Antibody Submission form, visit: nanostring.com/cosmx-pbs/

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