Quick Reference Guide

GeoMx Semi-Automated Slide Preparation: Protein FFPE

MAN-10139-04

Quick Reference Guides are not a substitute for the User Manuals. Please refer to the User Manuals until you are an experienced user.

1 Prepare Reagents

The following reagents are supplied by NanoString:

Kit	Reagents	Lot Number
GeoMx DSP Protein Slide Prep Kit for FFPE	Buffer S (4°C/RT) Buffer W (4°C)	
GeoMx Morphology Kit for Protein	Morphology markers (4°C) Nuclear Stain—SYTO 13 (-20°C)	
GeoMx Core & optional Module Kits for NGS or nCounter readout	Antibody (Ab) Mix (-80°C)	

For nCounter assays: Not all nCounter protein modules are compatible with one another. Each module is assigned a Probe R space. **Do not combine two modules with a common Probe R space** (i.e. same Probe R_number) in the same experimental run, or the data cannot be interpreted. Instead, use Substitute Probe R (available from NanoString). See nCounter protein modules' Probe R designations in the Appendix of the **GeoMx DSP Manual Slide Preparation User Manual** and use the planning grid at right, if needed.

The following reagents are not supplied by NanoString:

Reagents	
BOND Dewax Solution, 1 L	DEPC-treated water
BOND Epitope Retrieval 1, 1 L	1X TBS
BOND Wash Solution 10X Concentrate, 1 L (Dilute to 1X with DEPC-treated water)	1X TBS-T
4% PFA 	RNase AWAY®

R #	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
Core/ Module to be used									

See the **GeoMx DSP Automated Slide Preparation User Manual (MAN-10151)** for more information.

2 Prepare Samples

1. Use Apex BOND Adhesive slides. Ensure your tissue sample fits inside the **Scan Area** of the slide (green, in diagram at right) and within the white dots of the BOND slide. Tissue may not overlap gasket (blue) or tip calibration area (red). Important: If the tissue sample is outside of this area, the GeoMx will not process the slide.

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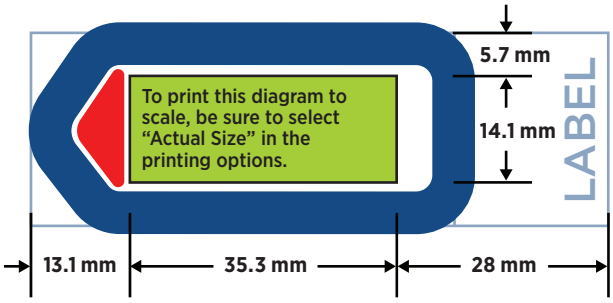
2. **Bake slides** at 60°C for 30 minutes to 3 hours.



3 Set up the BOND RX/RX^m

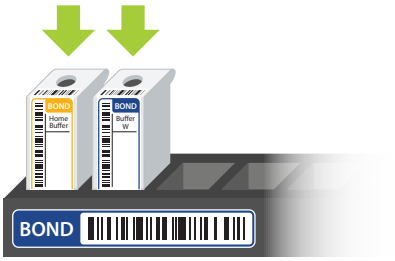
To set up the BOND RX/RX^m Semi-Automated Protein Protocol for the first time, refer to the GeoMx DSP Automated Slide Preparation User Manual (MAN-10151) for instructions.

Day 1 (continued)

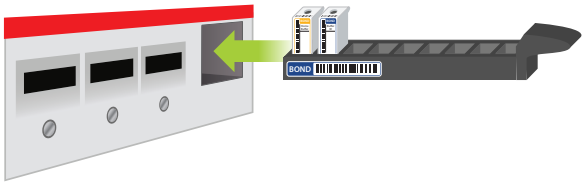
4 Start a Run on the BOND RX/RX^{m}

1. Fill the 30 mL Home Buffer container with 1X Bond Wash Buffer and place it in slot 1 of the Bond Research Detection System.

2. Fill the Titration Container insert with Buffer W and place in slot 2 of the Bond Research Detection System (150 µL per slide + 350 µL of dead volume).

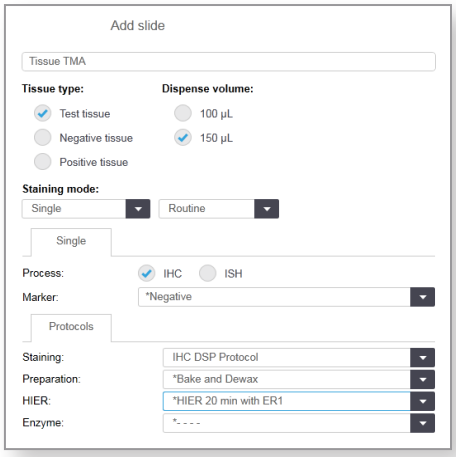


3. Load the Bond Research Detection System into the BOND RX/RX^{m}.



4. From Slide Setup, select Add Study. Enter study info; dispense volume 150 µL; Preparation protocol *Bake and Dewax. Select OK.

5. Select Add Slide. Enter slide information as shown below, then click Add slide.

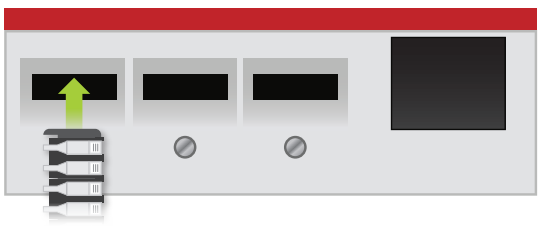


6. Print and attach labels to the slides.

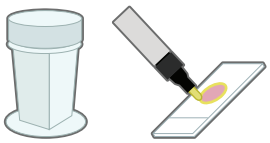
7. Load slides with covertiles into the slide tray. Ensure positioning is correct.

5 Load and Run your Slides

1. Load and run your slides. The run will take approximately 3 hours.



2. When run is complete, remove covertiles from one slide at a time and place slide in 1X PBS. Remove excess buffer and draw a closed barrier with a hydrophobic pen. DO NOT let slides dry out.



1X PBS

Day 1 (continued)

6 Primary Antibody Incubation

Thaw Ab mix, keeping the aliquot on ice and protected from light. Do not vortex. **Make working antibody solution** (see table). Handling one slide at a time, **blot excess buffer** and place slide in humidity chamber. **Cover tissue with working Ab solution**. Make sure the entire tissue is covered (~ 200 µL per slide). **Incubate overnight** in closed humidity chamber at 4°C, protected from light.

Humidity Chamber
Temp: 4°C
Time: Overnight

Working Ab solution for NGS or nCounter assays (n = number of slides):

Core Ab	Module Ab 1	Module Ab 2	Other Modules*	Morph Marker 1	Morph Marker 2	Other Markers	Buffer W	Total Volume
(8 µL x n) _____	(8 µL x n) + _____	(8 µL x n) + _____	...	(5 µL x n) + _____	(5 µL x n) + _____	...	(up to 200 µL)** + _____	(200 µL x n) = _____

*If adding custom barcoded Ab, see Appendix V of the GeoMx DSP Manual Slide Preparation User Manual for instructions.
**If a different number of module Ab or morphology markers are used, Buffer W volume can be adjusted; final volume = 200 µL/slide.

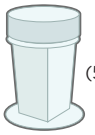
Day 2

7 Post-Fix

Staining jars should be thoroughly cleaned with RNase AWAY before use. Handling one slide at a time, wash in 1X TBS-T (see right for time and repetitions). Tap slide on paper towels to remove excess solution. Place slide in humidity chamber. Cover tissue with 4% PFA (~200 µL per slide). Incubate 30 minutes at room temp in closed humidity chamber. Wash with 1X TBS-T (see right).

1X TBS-T
3 washes
(10 min each)
☐☐☐

4% PFA in Humidity Chamber
Room Temp
Time: 30 min



1X TBS-T
2 washes,
(5 min each)
☐☐

8 Nuclei Staining

Picofuge thawed SYTO 13 1 min; do not pipette from the bottom of the vial. Dilute 1:10 in 1X TBS then mix by pipetting up and down. Handling one slide at a time, place in humidity chamber. Cover tissue with diluted SYTO 13 (~200 µL per slide). Incubate 15 minutes at room temp in closed humidity chamber. Dip slide into fresh 1X TBS-T, then transfer it to fresh 1X TBS-T. Scrape off hydrophobic pen on one slide at a time with a razor blade. DO NOT allow slide to dry out. Store in 1X TBS-T.

Nuclear stain in Humidity Chamber
Room Temp
Time: 15 min

1X TBS-T
2 washes, then store
☐☐

9 Additional resources in GeoMx DSP Automated Slide Preparation User Manual (MAN-10151)

- Slide storage guidelines
- Fresh frozen and fixed frozen sample preparation
- Alternative morphology marker methods
- Troubleshooting

For more information, please visit nanostring.com

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