Quick Reference Guide

GeoMx® Manual Slide Preparation: RNA FFPE

MAN-10130-06

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

Day 1

1 Reagent preparation

The following reagents are supplied by NanoString:

Kit	Reagents	Lot Number
GeoMx® DSP RNA Slide Prep Kit for FFPE	Buffer R (4°C), Buffer S (4°C/RT), Buffer W (4°C)	
GeoMx Morphology Kit for RNA	Morphology markers (4°C) Nuclear Stain—SYTO 13 (-20°C)	
GeoMx RNA Atlas or Assay for NGS or nCounter readout	Probe Mix (-20°C)	

The following reagents are not supplied by NanoString:

Reagents			
RNase AWAY®	1x Tris EDTA (pH 9)	DEPC-treated water	NBF Stop buffer
1x PBS (pH 7.4)	2x SSC	4x SSC	2x SSC-T
10% NBF	100% Formamide	Proteinase K	CitriSolv
100% & 95% Ethanol			

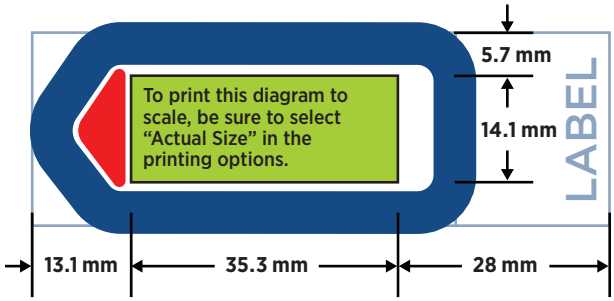
See the **GeoMx DSP Manual Slide Preparation User Manual (MAN-10150)** for more information.

2 Tissue preparation

1. Ensure your tissue sample fits inside the Scan Area of the slide (green, in diagram below). Tissue may not overlap gasket (blue) or tip calibration area (red).

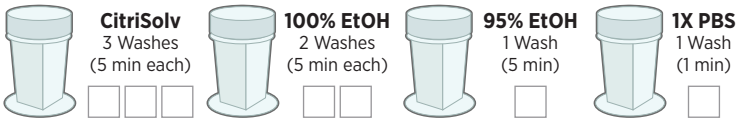
Important: If the tissue sample is outside of this area, the GeoMx will not process the slide.

2. **Bake slides** at 60°C for 30 min to 3 hr.



3 Deparaffinize and rehydrate FFPE tissue sections (31 minutes)

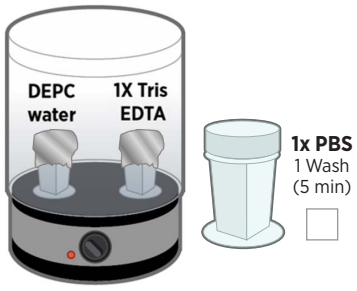
Preheat the steamer (with two loosely-covered staining jars – one with 1X Tris-EDTA (pH 9) and one with DEPC-treated water) **and water bath** (with ProK solution) for later use. **Wash slides** in staining jars (see right for recommended wash times and repetitions).



4 Target retrieval (15-25 minutes)

Ensure the **1X Tris-EDTA is ~99°C** before beginning. Uncover jars and dip slides in DEPC-treated water 10 sec. Transfer to 1X Tris-EDTA. **Incubate 20 min** (or 15 min for tonsil or if testing new tissue type - see table).

Tissue Type	Conditions
Breast	20 min
Cell pellets	Use hot plate*
Colorectal	20 min
Melanoma	20 min
Mouse tissue	20 min
NSCLC	20 min
Prostate tumor	20 min
Tonsil	15 min



*Use hot plate or water bath to heat buffer to 85°C, retrieve for 15 min.

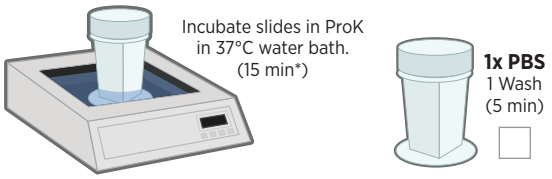
5 Expose RNA targets (10-30 minutes)

Place slides in preheated staining jar containing ProK (default 1µg/mL; see alternatives by tissue type in table). **Incubate** in 37°C water bath for 15 min or as indicated in table.

Proceed to next step immediately.

Table 2

Tissue Type	Conditions
Breast	0.1 µg/mL, 15 min
Cell pellets	1 µg/mL, 5 min
Colorectal	1 µg/mL, 15 min
Melanoma	1 µg/mL, 15 min
Mouse tissue	1 µg/mL, 15 min
NSCLC	1 µg/mL, 15 min
Prostate Tumor	1 µg/mL, 15 min
Tonsil	1 µg/mL, 15 min



Day 1 (Continued)

6 Post-fix preservation (20 min)

Wash slides in NBF, stop buffer, and PBS. See figure below for wash repetitions and times.

10% NBF
1 Wash (5 min)
☐

NBF Stop Buffer
2 Washes (5 min each)
☐☐

1x PBS
1 Wash (5 min)
☐

7 Hybridization (overnight)

Clean hybridization chamber with RNase AWAY and prepare chamber with damp Kimwipes. **Prepare reagents:** Warm Buffer R to room temp before opening and bring RNA detection probes to room temp to allow more accurate pipetting of viscous solution. Mix RNA probes by flicking and spinning down tube. **Make hybridization solution following Table 3 for NGS assays or Table 4 for nCounter assays.**

Table 3: Hybridization solution for NGS Assays (n = number of slides)

Panel Configuration	Buffer R	Atlas Probe Mix	Custom Assay 1	Custom Assay 2	DEPC-treated H_2O	Final volume
CTA or WTA	200 µL x n	25 µL x n	0	0	25 µL x n	250 µL x n
CTA or WTA + 1 Custom Assay	200 µL x n	25 µL x n	12.5 µL x n	0	12.5 µL x n	250 µL x n
CTA or WTA + 2 Custom Assays	200 µL x n	25 µL x n	12.5 µL x n	12.5 µL x n	0	250 µL x n

For custom assays without CTA or WTA, see guidance in **GeoMx DSP Manual Sample Preparation User Manual (MAN-10150).**

Table 4: Hybridization solution for nCounter Assays (n = number of slides)

Panel Configuration	Buffer R	Immune Pathways Panel	Custom Assay	DEPC-treated H_2O	Final volume
RNA Pathways Panel	200 µL x n	37.5 µL x n	0	12.5 µL x n	250 µL x n
RNA Pathways Panel +1 Custom Assay	200 µL x n	37.5 µL x n	12.5 µL x n	0	250 µL x n

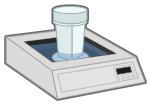
Handling one slide at a time, wipe excess PBS and place in hyb chamber. Add 200 µL hyb solution and gently apply a Hybrislip coverslip, avoiding air bubbles. Repeat for all slides. Place hyb chamber in oven to hybridize overnight at 37°C.

Day 2

8 Perform stringent washes (1 hour)

Warm 100% formamide to room temp before opening. Staining jars should be cleaned with RNase AWAY before use. **Make Stringent Wash** by mixing equal parts 100% formamide and 4X SSC. Fill two staining jars with Stringent Wash and **preheat them to 37°C** in water bath.

Dip slides in 2X SSC allowing coverslip to slide off. DO NOT FORCE coverslip removal. If needed, place slides in 2X SSC-T to help remove coverslip. **Proceed to washes** as shown at right.



Stringent Wash Solution at 37°C
2 Washes
(25 min each)
☐☐

2X SSC
2 Washes
(2 min each)
☐☐

9 Block and add morphology markers (100 min)

Block:

Cover tissue with up to 200 µL Buffer W for 30 min in the humidity chamber at room temp.

Make morphology marker solution for NGS or nCounter assays (n= number of slides)

Note: Flick and spin reagents first.

SYTO13	Morph Marker1	Morph Marker2	Other Markers	Buffer W	Total Volume
(22 µL x n)*	(5.5 µL x n) + _____	(5.5 µL x n) + _____	…	(187 µL x n)** + _____	(220 µL x n) = _____

* Picofuge 1 minute & avoid pipetting from bottom of vial.
**If a different number of morphology markers are used, Buffer W volume can be adjusted; final volume = 220 µL/slide.

Cover with up to 200 µL morph markers for 1 hour in the humidity chamber at room temp.

2X SSC
2 Washes (5 min each)
☐☐

10 Additional Resources

GeoMx DSP Manual Slide Preparation User Manual (MAN-10150)

- 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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