

G4X Assay Quick Reference Card

Workflow

- Transcriptomic
 Multiomic

Flow Cell Type

- 4 lane flow cell
 2 lane flow cell

Number of Lanes

- Add Steps Checkboxes
 Add Buffer Checkboxes
 Transcriptomic add-on

Print

The G4X™ Assay Quick Reference Card (QRC) contains concise instructions for the off-instrument assay steps for proteomics and transcriptomics on the G4X. This QRC is intended for experienced instrument users. For more information and detailed instructions, see the G4X Sample Preparation Guide.

For all steps, make sure to leave fluid in during incubation or wash time unless otherwise noted.

This G4X™ Assay Quick Reference Card (QRC) is a supplement to the existing G4X™ Sample Preparation Guide (Rev. 5).

2. Reconstitute Tissue and Targets

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2.1 Bake Sample Slide

1. Place TissuGrip™ sample slides on a glass slide staining rack. If multiple slides are prepared at once, ensure all slides face the same direction and are spaced to prevent tissue sections from touching each other or the dividers in the rack.
2. Place the staining rack in a 60°C oven for 20 minutes. Keep oven door shut for the full incubation to avoid temperature fluctuations.
3. Remove the staining rack from the oven and allow to cool down to room temperature for 10 minutes. Do not remove slides from the staining rack.

2.2 Deparaffinization

1. Prepare 6 glass staining dishes for slides:

Staining Dish	Reagent	Volume	Notes
1	Xylene (first incubation)	250 mL	First xylene incubation
2	Xylene (second incubation)	250 mL	Second xylene incubation
3	100% ethanol (first incubation)	250 mL	First ethanol incubation
4	100% ethanol (second incubation)	250 mL	Second ethanol incubation
5	95% ethanol	250 mL	237.5 mL 100% ethanol + 12.5 mL DEPC-treated water
6	70% ethanol	250 mL	175 mL 100% ethanol + 75 mL DEPC-treated water

NOTE: Reagents in the staining dishes can be reused for up to 1 week or 50 slides, whichever comes first. Keep staining dishes covered when not in use to prevent evaporation. Prepare fresh solutions if the solutions cannot be reused anymore.

2. Submerge the glass staining rack in the following staining dishes for the specified incubation times. Glass slides should be fully submerged in solvent. When transferring between dishes, let the solution drain briefly from the glass staining rack. This will minimize carry-over and keep the reagents fresh.

NOTE: HistoClearII may be used in place of xylene, for more information, refer to the *G4X Sample Preparation Guide*. If using HistoClear II, Incubate slides in HistoClear II for **10 minutes** each for optimal performance.

Staining Dish	Reagent	Incubation Time
1	Xylene (first incubation)	5 min
2	Xylene (second incubation)	5 min
3	100% ethanol (first incubation)	3 min
4	100% ethanol (second incubation)	3 min
5	95% ethanol	3 min
6	70% ethanol	3 min

3. Remove staining rack from the staining dish and place on a paper towel or other absorbent material to remove excess ethanol from the rack.
4. Fill the wells in a 4-well plate with 5 mL of DEPC-treated water. You will need a well for each slide that is being prepared.
5. Remove the slides from the staining rack and transfer each slide into a separate well of the 4-well plate. Let flow cell sit for 30 seconds to wash.
6. Take a picture of your slide(s) to note the location of your

tissues (use a dark background for best visibility).

2.3 Perform Target Retrieval

1. Mark the front of the Coplin Jar with a marker. This will help placing the slides in an orientation that avoids the tissue side of the slides touching the dividers in the Coplin Jar.
2. Make 70 mL of Target Retrieval Solution in each Coplin Jar. Seal the Coplin Jar with lid and mix by inverting 10 times. **Let solution sit for at least 2 minutes** so the bubbles have time to disappear.

Target Retrieval Solution	Volume per Coplin Jar
Concentrated Target Retrieval Solution	7.0 mL
DEPC-treated water	63.0 mL
Total	70.0 mL

3. Open the Coplin Jar and carefully insert the glass slides into the dividers of the Coplin Jar, at most two slides per jar. Make sure the slide are separated from one another and lean back the Coplin Jar to prevent the slides from falling forward and have the tissue touch the dividers. Tightly secure the lid.
4. Once the pressure cooker is pre-heated and boiling, add Coplin Jar containing slides and Target Retrieval Solution directly on bottom of the pressure cooker and tip in same direction (using pot curvature) to keep slides from touching dividers. You can put 3 jars in one pressure cooker.
5. Close pressure cooker lid and put on high pressure for 30 minutes. The time count will not start until the pressure cooker reaches pressure.
6. When the 30 minutes are over, immediately vent the pressure cooker using **Fast Release**.
7. Once venting is complete, put on heat-resistant autoclave gloves and remove the Coplin Jar.
8. Allow the Coplin Jar to cool on the bench top for 20 minutes.
9. Prepare a 4-well plate with the following reagents in each well. Remove the slide from Coplin jar and using forceps, move the slide to the first well with the tissues facing up. Gently agitate the 4-well plate and let it incubate for the indicated incubation times before moving it to the next well.

Well in 4-well plate	Reagent (5 mL)	Incubation Time
1	DEPC-treated water	1 min
2	DEPC-treated water	1 min
3	DEPC-treated water	1 min
4	100% ethanol	30 s

10. Dry the slide at room temperature to remove all traces of ethanol. There should be no visible droplets on the slide and the tissue sections should be opaque, about 10 minutes.



CRITICAL STEP

- Examine slide after drying. If salts, water marks, or other stains are observed on slide, repeat wash steps in step and dry slide.
- Slides must be completely dry prior to assembly.

3. Prepare Flow Cell

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3.1 Assemble Flow Cell

1. Assemble flow cell using the flow cell assembler and flow cell press.

3.2 Install Flow Cell in Pipette Aid

1. Install assembled flow cell in the pipette aid. Ensure that pipette aid base is clean prior to installation.
2. Empty waste container and dispose of waste in hazardous waste.
3. Install pipette aid in the caddy and assemble all parts into the caddy.
4. Perform the following washes for each lane:

Clear lane?	Volume	Reagent	Time	Temp
a	500 μ L	Wash Buffer	No incubation	RT
b	500 μ L	Wash Buffer	No incubation	RT

4. Antibody Binding

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4.1 Tissue Passivation

4.1.1 Prepare Buffers

1. Spin down Tissue Passivation Reaction Buffer and Antibody Binding Reaction Buffer tubes in a mini centrifuge to ensure that no material is left in the tube caps.
2. Pipette 1400 μ L of Tissue Passivation Reaction Buffer into a Protein low bind tube. Similarly, pipette 345 μ L of Antibody Binding Reaction Buffer into a **separate** Protein low bind tube.
3. Centrifuge both tubes in a centrifuge set to 4°C for 10 minutes at 10,000xg. Be aware of the orientation of the tubes when placing into the centrifuge as a pellet may not be visible.
4. After centrifugation, transfer 1247 μ L of Tissue Passivation Reaction Buffer into a new Protein low bind tube and transfer 330 μ L of Antibody Binding Reaction Buffer into a **separate** Protein low bind tube. Avoid pulling directly from the bottom of the tube to not disturb a possible pellet.
5. Keep buffers on ice until ready to use.

4.1.1 Tissue Passivation Procedure

1. Prepare Tissue Passivation Working Mix by adding RNase inhibitor 2 to the centrifuged Tissue Passivation Reaction Buffer. Pipette up and down 10 times with a pipette to mix. **Do not vortex**. Keep buffer on ice until ready to use.

Tissue Passivation Working Mix	Volume
Tissue Passivation Reaction Buffer	1247.0 μ L
RNase Inhibitor 2	12.5 μ L
Total	1259.5 μL

2. Perform the following washes and incubations for each lane:

	Clear lane?	Volume	Reagent	Time	Temp
a	✓	70 μ L	Tissue Passivation Working Mix	30 min	RT



CRITICAL STEP

After this step, save the remainder of the Tissue Passivation Working Mix on ice as it will be used for the next step.

4.2 Bind Antibodies to Protein

1. Set heat block to 37°C.
2. Prepare Antibody Binding Working Mix on ice by adding RNase inhibitor 2 to the centrifuged Antibody Binding Reaction Buffer. Pipette up and down 10 times with a pipette to mix. **Do not vortex**. Keep mix on ice until ready to use.

Antibody Binding Working Mix	Volume
Antibody Binding Reaction Buffer	330.0 µL
RNase Inhibitor 2	3.3 µL
Total	333.3 µL


NOTE: If using a custom protein panel, use [G4X Custom Antibody Calculator](#).

3. Perform the following washes and incubations for each lane:

	Clear lane?	Volume	Reagent	Time	Temp	Notes
a	✓	70 µL	Antibody Binding Working Mix	2 h	37°C	
b	✓	70 µL	Tissue Passivation Working Mix	15 min	37°C	Switch heat block to 45°C after incubation
c		200 µL	Wash Buffer	5 min	RT	
d		200 µL	Wash Buffer	5 min	RT	

4.3 Protein Conditioning

1. Perform the following washes and incubations for each lane:

	Clear lane?	Volume	Reagent	Time	Temp	Notes
a	✓	70 µL	Protein Conditioning Solution	10 min	45°C	
b	✓	200 µL	Wash Buffer	5 min	RT	
c	✓	200 µL	Wash Buffer	5 min	RT	



CRITICAL STEP

Excessive bubbles may form when flowing Protein Conditioning Solution. If excessive bubbles do form, use a manual pipette to quickly push more Protein Conditioning Solution through the lane and dislodge the bubbles.

5. Incorporate Padlock Probes

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5.1 Hybridize Transcript Padlock Probes

1. Set a heat block to 45°C.
2. Vortex PLP Reaction Buffer for 60 seconds.
3. Prepare fresh Transcript PLP Working Mix. Combine all solutions together and vortex to mix.

Transcript PLP Working Mix	Total Volume	Volume per FC	Volume per Lane
PLP Reaction Buffer	331.6 μ L	331.6 μ L	82.9 μ L
Transcript Panel	63.0 μ L	63.0 μ L	15.8 μ L
RNase Inhibitor 1	31.5 μ L	31.5 μ L	7.9 μ L
DEPC-treated water	203.9 μ L	203.9 μ L	51.0 μ L
Total	630 μL	630 μL	157.5 μL

4. Perform the following washes and incubations for each lane:



CRITICAL STEP

After this step, save the remainder of the Hyb Wash Buffer at 2°C to 8°C for the next protocol step.

Clear lane?	Volume	Reagent	Temp	Time	Notes
a	500 μ L	Hyb Wash Buffer	RT	No incubation	
b	70 μ L	Transcript PLP Working Mix	45°C	Overnight (14 h–20 h)	Install Pipette Aid Plugs

NOTE: Bubbles forming on the borders of the lanes during the overnight incubation is common and no reason for concern.



CRITICAL STEP

Dispose of the pipette aid plug after the overnight incubation.

5. Perform the following washes for each lane:

Clear lane?	Volume	Reagent	Temp	Time	Notes
a	200 µL	Hyb Wash Buffer	45°C	5 min	
b	200 µL	Hyb Wash Buffer	45°C	5 min	
c	200 µL	Wash Buffer	RT	5 min	
d	200 µL	Wash Buffer	RT	5 min	

6. Prepare PLP Conditioning 1 Solution and PLP Conditioning 2 Solution on ice. Pipette up and down 10 times to mix. **Do not vortex**. Keep both solutions on ice until ready to use.

PLP Conditioning 1 Solution		PLP Conditioning 2 Solution	
	Volume		Volume
PLP Conditioning Buffer	623.7 µL	PLP Conditioning Buffer	604.8 µL
RNase Inhibitor 2	6.3 µL	RNase Inhibitor 2	6.3 µL
Total	630.0 µL	PLP Conditioning 2 Spike-In	18.9 µL
		Total	630.0 µL

7. Perform the following incubations and washes for each lane:

Clear lane?	Volume	Reagent	Time	Temp	Notes
a ✓	70 µL	PLP Conditioning 1 Solution	No incubation	RT	
b ✓	70 µL	PLP Conditioning 2 Solution	15 min	45°C	
c	500 µL	Wash Buffer	No incubation	RT	

8. Prepare Protein PLP Working Mix on ice. Pipette up and down 10 times to mix. **Do not vortex**. Keep both solutions on ice until ready to use.

Protein PLP Working Mix	Volume
DEPC-treated water	253.7 μ L
PLP Reaction Buffer	331.6 μ L
G4X IO Proteomic Panel	13.2 μ L
RNase Inhibitor 1	31.5 μ L
Total	630.0 μL

9. Perform the following incubations and washes for each lane:



CRITICAL STEP

The following step requires accurate execution and timing.

Clear lane?	Volume	Reagent	Time	Temp	Notes
a	500 μ L	Hyb Wash Buffer	No incubation	RT	
b ✓	70 μ L	Protein PLP Working Mix	30 min	45°C	Switch heat block temperature to 37°C after incubation
c	500 μ L	Wash Buffer	No incubation	RT	

5.2 Ligate Padlock Probes

1. Set a heat block to 37°C.
2. Vortex and spin down both tubes of Ligation Reaction Buffer.
3. Prepare Ligation Priming Buffer and Ligation Working Mix on ice. Pipette up and down 10 times to mix. **Do not vortex.** Keep both solutions on ice until ready to use.

Ligation Priming Buffer		Ligation Working Mix	
	Volume		Volume
Ligation Reaction Buffer	623.7 µL	Ligation Reaction Buffer	603.5 µL
RNase Inhibitor 2	6.3 µL	RNase Inhibitor 2	6.3 µL
Total	630.0 µL	Ligase	20.2 µL
		Total	630.0 µL

4. Perform the following washes and incubations for each lane:



CRITICAL STEP

The following step requires accurate execution and timing.

	Clear lane?	Volume	Reagent	Time	Temp	Notes
a	✓	70 µL	Ligation Priming Buffer	No incubation	RT	Ensure all remaining liquid from the previous buffer is removed when clearing out.
b	✓	70 µL	Ligation Working Mix	30 min	37°C	
c		500 µL	Wash Buffer	No incubation	RT	

5.3 Amplify Padlock Probes

1. Prepare AMP 1 Working Mix. Pipette up and down 10 times to mix. **Do not vortex**. Keep working mix on ice until ready to use.

AMP 1 Working Mix	Volume
AMP 1 Reaction Buffer	623.7 μ L
RNase Inhibitor 2	6.3 μ L
Total	630.0 μL

2. Perform the following washes and incubations for each lane:



CRITICAL STEP

The following step requires accurate execution and timing.

Clear lane?	Volume	Reagent	Time	Temp	Notes
a	500 μ L	HYB Wash Buffer	No incubation	RT	
b	✓ 70 μ L	AMP 1 Working Mix	10 min	37°C	

3. Vortex and spin down a tube of AMP 2 Reaction Buffer and AMP 3 reaction buffer.
4. Prepare AMP 2 Working Mix and AMP 3 Working Mix. Pipette up and down 10 times to mix. **Do not vortex**. Keep both mixes on ice until ready to use.

AMP 2 Working Mix	Volume	AMP 3 Working Mix	Volume
AMP 2 Reaction Buffer	1134.0 μ L	AMP 3 Reaction Buffer	623.7 μ L
RNase Inhibitor 2	12.6 μ L	RNase Inhibitor 2	6.3 μ L
AMP Enzyme	113.4 μ L	Total	630.0 μL
Total	1260.0 μL		

5. Perform the following washes and incubations for each lane:



CRITICAL STEP

The following step requires accurate execution and timing.

Clear lane?	Volume	Reagent	Time	Temp	Notes
a	200 μ L	HYB Wash Buffer	5 min	37°C	
b	200 μ L	HYB Wash Buffer	5 min	37°C	
c	500 μ L	Wash Buffer	No incubation	RT	Switch heat block temperature to 33°C
d	✓ 70 μ L	AMP 2 Working Mix	15 min	33°C	
e	✓ 70 μ L	AMP 2 Working Mix	15 min	33°C	Switch heat block to 37°C after incubation
f	✓ 70 μ L	AMP 3 Working Mix	3 h	37°C	
g	500 μ L	PBS-E	No incubation	RT	

6. Sequencing Conditioning

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6.1 Sequencing Conditioning

1. Prepare Conditioning 1 Solution.

Conditioning 1 Solution	Volume
Concentrated Conditioning 1 Solution	63.0 μ L
PBS-T2	567.0 μ L
Total	630.0 μL



CRITICAL STEP

Conditioning 1 Solution needs to be made fresh prior to the incubations, make solution right before use.

2. Perform the following washes and incubations for each lane:

	Clear lane?	Volume	Reagent	Time	Temp	Notes
a	✓	70 µL	Conditioning 1 Solution	60 min	RT	
b		500 µL	Wash Buffer	No incubation	RT	
c	✓	70 µL	Conditioning 2 Solution	30 min	RT	Set heat block to 60°C.
d		500 µL	Wash Buffer	No incubation	RT	
e		200 µL	Saline Rinse	10 min	60°C	
f		500 µL	Wash Buffer	No incubation	RT	

3. Perform the following washes and incubations for each lane:

	Clear lane?	Volume	Reagent	Time	Temp	Notes
a	✓	70 µL	Conditioning 3 Solution	15 min	60°C	
b		500 µL	Wash Buffer	No incubation	RT	
c	✓	70 µL	Conditioning 4 Solution	15 min	60°C	Start preparing Conditioning 5 Solution.
d		500 µL	Wash Buffer	No incubation	RT	

4. Prepare fresh Conditioning 5 Solution. Add PBS-T2 to the Conditioning 5 Solid tube to suspend the pellet. Tape the tube to vortexer and vortex solution at low speed for 15 minutes. Ensure that all solids are dissolved.

NOTE: A rotating mixer may be used to mix the solution as an alternative to a vortexer. Mix solution for 15 minutes on the rotating mixer and ensure that all solids are dissolved.

Conditioning Solution 5	Volume
Conditioning 5 Solid	-
PBS-T2	1600 μ L
Total	1600 μL



CRITICAL STEP

Conditioning 5 Solution needs to be made fresh prior to the incubation, make solution right before use.

5. Perform the following washes and incubations for each lane:

	Clear lane?	Volume	Reagent	Time	Temp	Notes
a	✓	70 μ L	Conditioning 5 Solution	15 min	60°C	
b		500 μ L	Wash Buffer	No incubation	RT	

6.2 Add Focusing Beads

- Vortex the provided Focusing Beads solution for 30 seconds immediately before loading Focusing Beads to the flow cell.
- Pipette the following amounts of Focusing Beads into each lane:



CRITICAL STEP

Pipette Focusing Bead solution immediately after vortexing. If doing multiple flow cells at a time, vortex Focusing Bead solution between flow cells. Pipette Focusing Bead solution from the bottom of the tube.

	Clear lane?	Volume	Reagent	Time	Temperature
a	✓	70 μ L	Focusing Bead	6 min	RT

- After incubation, clear the lane to remove as much Focusing Bead solution as possible.



CRITICAL STEP

Ensure Focusing Bead solution is mostly removed before washing with Wash Buffer to avoid generating excessive bubbles. If excessive bubbles do form, wash the lanes with Wash Buffer multiple times to fully wet them before proceeding to the next step.

- Perform the following washes for each lane:

	Clear lane?	Volume	Reagent	Time	Temperature
a	✓	500 μ L	Wash Buffer	No incubation	RT
b		500 μ L	Wash Buffer	No incubation	RT

- Remove flow cell from pipette aid, tape up flow cell and refer to the G4X Spatial Sequencer Guide for sequencing.

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