User Guide | CG001699 | Rev A

Atera
Training Kit Supplemental User Guide

For use with:

Atera Training Bundle PN-1001196

Atera Assay Prep Reagents, 2 rxns PN-1000975

Notices

Document Number

CG001699 | Rev A

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Document Revision Summary

Document Number

CG001699

Title

Atera Training Kit Supplemental User Guide

Revision

Rev A

Revision Date

October 16, 2026

Description of Changes

N/A

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Training Objectives

The following are Atera training objectives. By the end of training, users will be able to prepare tissue samples, start an Atera Instrument run, and obtain data outputs.

Tissue Preparation	
Sample Quality	
☐	Review how tissue fixation method and duration affect assay performance.
☐	Review how H&E staining is used to assess tissue morphology and quality.
☐	Review how DV200 is used to assess RNA integrity in FFPE samples.
☐	Identify when to exclude a sample before starting the assay based on quality assessment, block condition, and slide storage.
Sample Processing	
☐	Review best practices for tissue block handling, storage, and tissue sectioning.
☐	Identify tested slides for the Atera assay.
☐	Review correct storage conditions and duration for slides with tissue sections.
☐	Identify appropriate sections for the Atera assay.

Atera Cassette and Insert Use	
Cassette Assembly	
☐	Demonstrate correct cassette assembly and disassembly technique. Consult Atera Cassette Assembly, Disassembly, and Use, Quick Reference Card (Document CG001674).
☐	Review correct lid application.
☐	Identify when the Atera Cassette Insert is used and review correct insert application.
☐	Review possible cassette, lid, and insert failure modes.
Pipetting Technique	
☐	Demonstrate correct pipetting technique for the Atera Cassette, the Atera Cassette Insert, and specific techniques for Amp Mix.
☐	Demonstrate correct pipetting technique for the Atera Cassette Insert, and specific techniques for Amp Mix.
☐	Ensure slides stay hydrated throughout the workflow, except when noted otherwise.

Assay Workflow Execution	
Thermal Cycler Setup	
☐	Identify supported thermal cyclers.
☐	Program or transfer all thermal cycler protocols.
☐	Review step time, temperature, and thermal cycler lid temperature for each relevant protocol step.
☐	Review acceptable and unacceptable cassette condensation during the workflow.
Workflow Execution	
☐	Review best practices for processing two or four slides, and considerations for preparing odd numbers of slides (not recommended).
☐	Review reagent preparation instructions, including thawing time and temperature, for all reagents.
☐	Review appropriate safe stopping points in the workflow.
☐	Review importance of wash steps to data quality.
☐	Review when Atera Instrument reagents need to be thawed.
☐	Execute the workflow using the appropriate tissue preparation Handbook and User Guide.

Atera Instrument	
Instrument Orientation	
☐	Identify the instrument power button, breaker switch, door, keyboard, and USB ports.
☐	Review RFID tracking system.
☐	Upload JSON files to the Atera Instrument.
☐	Locate the data folder and explain data transfer.
☐	Run a readiness test.
☐	Review how to enable remote access for 10x Genomics Technical Support.
☐	Review how to restart the Atera Instrument.
☐	Verify cassette integrity before loading, ensuring no leaks and that the lids are removed.
☐	Load both Atera Training Slides onto the Atera Instrument.
Pouch Loading	
☐	Review pouch storage, thawing, and inversion requirements.
☐	Load pouches, confirming pouch caps are removed and pouches are fully seated.
Plate Loading	
☐	Thaw plates correctly before loading.
☐	Prepare plates correctly before loading.
☐	Review assay-specific plate compatibility and demonstrate plate positioning.
☐	Load plates in the correct orientation.

Training Run	
Initiating a Run	
☐	Perform region selection and naming.
☐	Initiate a training run using the Atera Instrument Software.
Unloading & Maintenance	
☐	Review maximum time cassettes can remain on instrument before unloading.
☐	Review cleanup procedure, including duration and use of the cleanup cassette (includes cleanup cassette cleaning and storage), and reagent pouch disposal.
☐	Review maintenance wash procedure, including duration and use of the maintenance wash bottles (includes wash bottle cleaning and storage).
☐	Review unloading the Atera Instrument.
Reviewing Run Outputs & Data	
☐	Review data transfer options: network, cloud storage, or USB (not recommended).
☐	Identify expected outputs of a completed Atera training run.
☐	Identify the location of the analysis summary file.
☐	Interpret key quality metrics in the analysis summary.

Atera Bundles

Atera Bundles	Part Number	Components	#	Component PN	Storage
Atera Training Bundle	1001196	Atera FFPE Sample Prep Reagents (2 reactions)	1	1001112	–20°C
		Atera Assay Prep Reagents (2 reactions)	1	1000975	–20°C
		Atera Select Human Multi-Tissue Panel (A) (2 reactions)	1	1000977	–20°C
		Atera Training Slides	1	1001144	4°C
		Atera Cassette Kit (2 cassettes)	1	1001153	Ambient
		Atera Plate 1 (1 run, 2 slides)	1	1000971	–20°C
		Atera Pouch 123 Bundle (Universal)	1	1001152	Ambient
		Atera Pouch 4 (Small)	1	1001150	–20°C

Atera Gene Expression Reagent Kits

The Atera Gene Expression workflow also requires Atera Hyb Buffer and Reducing Agent B (FFPE Kit), which can be found in the sample preparation kits. Consult the Atera Gene Expression – FFPE Tissue Preparation Handbook (Document CG001671) for more information.

Atera FFPE Sample Prep Reagents (2 reactions) PN-1001112

Consult SDS for handling and disposal information.

Atera FFPE Sample Prep Reagents (2 reactions)
PN-1001112
Shipped on dry ice; Store at –20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
●	FFPE Tissue Enhancer	2000798	●	Perm Enzyme B	3000553
●	Atera Reagent S	2001913	●	Atera Wash Enhancer	2001914
○	Reducing Agent B	2000087	○	Atera Hyb Buffer	2001879
-	empty	-	-	empty	-
-	empty	-	-	empty	-

Atera Assay Prep Reagents (2 reactions) PN-1000975

Consult SDS for handling and disposal information.

Atera Assay Prep Reagents (2 reactions)
PN-1000975
Shipped on dry ice; Store at –20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
○	Post-Hybridization Wash Buffer	2000395	●	Atera Ligation Buffer	2001880
●	Atera Enzyme A	2001915	●	Atera Enzyme B	2001916
●	Amplification Enhancer Buffer	2001234	●	Atera Amplification Enhancer	2001917
●	Amplification Enhancer Wash Buffer	2001236	●	Atera Amp Mix 1	2001976
●	Atera Amp Mix 2	2001881	●	Atera Signal Booster	2001947

Atera Cassette Kit (2 cassettes) PN-1001153

Atera Cassette Kit
PN-1001153
Shipped at ambient temperature
Store at ambient temperature

	#
Atera Cassette Top	2
Atera Cassette Bottom	2
Atera Cassette Insert	4
Atera Cassette Lid	2

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Atera Training Slides (2 slides) PN-1001144

Atera Training Slides, 2 slides
PN-1001144
Shipped on dry ice
Store at 4°C

	#	PN
Atera Training Slides (2 pack)	1	3003102

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Atera Plates and Pouches

Atera Plates	#	Kit PN	Component PN	Shipping and Storage
Atera Plate 1 (1 run, 2 slides)	1	1000971	2001614	Shipped on dry ice, store at −20°C

Atera Pouches	#	Kit PN	Component PN	Shipping and Storage
Atera Pouch 1 (Universal)	1	1001147	2001931	Shipped and stored at ambient temperature
Atera Pouch 2 (Universal)	1	1001148	2001932	Shipped and stored at ambient temperature
Atera Pouch 3 (Universal)	1	1001149	2001933	Shipped and stored at ambient temperature
Atera Pouch 4 (Small)	1	1001150	2001935	Shipped on dry ice, store at −20°C

Atera Training Slides

- Atera Training Slides contain two cell pellet sections, which are a combination of Jurkat and Raji cells.
- Before starting steps in the Atera Gene Expression – FFPE Tissue Preparation Handbook (Document CG001671), remove slides from packaging and equilibrate at room temperature for 5–10 min.

Atera Training Slide



Third-Party Items

Successful execution of this workflow requires third-party reagents, kits, and equipment in addition to those provided by 10x Genomics. Obtain all necessary third-party reagents and consumables before starting this workflow.

Consult the Atera Gene Expression Protocol Planner (Document CG001669) for a list of the following third-party items:

- Additional reagents, kits, and equipment
- Tested slides

10x Genomics has tested all items listed in the Protocol Planner. Unless otherwise noted, 10x Genomics recommends using these items to ensure optimal assay performance.



Substituting materials may adversely affect assay performance.

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Atera Accessory Practice

Atera Accessory Practice Overview

The Atera Gene Expression assay requires several accessories throughout the workflow. They are:

- Atera Cassettes
- Atera Cassette Lids
- Atera Cassette Insert
- Atera Thermocycler Adaptor

The Atera Cassette Assembly, Disassembly, and Use, Quick Reference Card (Document CG001674) is a companion document that details instructions on using all of the accessories above.

Before proceeding, review the following procedures:

- Cassette assembly and disassembly
- Lid application
- Reagent addition and removal from the Atera Cassettes, with or without the insert
- Locking the Thermocycler Adaptor in the angled position, and returning it to a flat position

Review the Quick Reference Card before starting this User Guide, as well as any other Atera Gene Expression Handbooks or User Guides.



Users cannot practice insert application and removal in advance. Keep inserts in their packaging until the assay. If desired, save inserts after the Atera assay is complete for future practice.

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Product Documentation

Documentation Overview

A variety of product documentation supports the Atera Gene Expression assay and Atera Instrument. Consult the 10x Genomics support website for the latest documentation, including an updated list of tested slides and thermal cyclers.

- CG001669 Atera Gene Expression Protocol Planner
  - Third-party items, reagents, tested slides, and thermal cyclers.
- CG001670 Atera Gene Expression – Fresh Frozen Tissue Preparation Handbook
  - Protocols for tissue freezing, embedding, sectioning, quality assessment, fixation, and pretreatment.
- CG001671 Atera Gene Expression – FFPE Tissue Preparation Handbook
  - Protocols for tissue fixation, embedding, quality assessment, sectioning, deparaffinization, and decrosslinking.
- CG001672 Atera Gene Expression User Guide
  - Protocols for probe hybridization, ligation, amplification, optional cell segmentation, ethanol drying, and preparation for loading the Atera Instrument.
- CG001673 Atera Instrument Operation User Guide
  - Instructions on Atera Instrument operation, maintenance, and troubleshooting.

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Training Schedule

Training Schedule Overview

Below are two examples of an Atera training schedule, which may be modified to accommodate specific needs. A Field Application Scientist will communicate final schedule details.

Pre-Training Call (Virtual)

Users will receive pre-training calls to prepare for on-site training and cover the workflow overview.

Three-Day Training (On Site)

Day 1: FFPE Tissue Preparation (9:00 AM–3:30 PM)

9:00 AM	**WET LAB**	Training kickoff; incubate slides at 60°C for 2 h; program thermal cyclers	CG001671
9:30 AM	**WET LAB**	Prepare buffers and solutions for deparaffinization	CG001671
10:20 AM	**WET LAB**	Cassette assembly practice	CG001671
11:15 AM	**WET LAB**	Deparaffinization	CG001671
12:30 PM	**WET LAB**	Decrosslinking	CG001671
	HANDS-OFF	*Lunch*	
12:40 PM	**WET LAB**	Thaw probe hybridization reagents *(During thermal cycler step)*	CG001672
1:30 PM	**WET LAB**	Prepare Probe Hybridization Mix and start Probe Hybridization protocol on thermal cycler	CG001672
1:45 PM	**HANDS-OFF**	*Probe Hybridization in progress (16–24 h; incubate overnight)*	CG001672
2:00 PM	**DISCUSSION**	Review Day 2 schedule *Optional: Review fresh frozen protocol*	CG001672
2:30 PM	**HANDS-OFF**	Break (if necessary)	
3:30 PM	**DISCUSSION**	Instrument walkthrough	CG001673

Day 2: Assay Workflow (9:00 AM–4:00 PM)

9:00 AM	WET LAB	Thaw Post-Hybridization Wash Buffer Retrieve cassettes and start Post Hybridization Wash Thaw Ligation reagents, prepare Ligation Mix and start Ligation protocol on thermal cycler	CG001672
10:00 AM	HANDS-OFF	*Ligation protocol in progress* Obtain and reagents for amplification	CG001672
10:45 AM	WET LAB	Prepare Amplification Enhancement Mix and start Amplification Enhancement protocol on thermal cycler	CG001672
11:30 AM	HANDS-OFF	*Amplification Enhancement protocol in progress* *Lunch and Review Cell Segmentation and Instrument Operation*	CG001672
1:00 PM	WET LAB	Add Amplification Mix and start Amplification protocol on thermal cycler	CG001672
1:30 PM	HANDS-OFF	*Amplification protocol in progress* *Review stopping points*	CG001672
3:00 PM	WET LAB	Post Amplification Wash	CG001672
3:30 PM	WET LAB	Signal Boost	CG001672
3:45 PM	WET LAB	Ethanol Drying and store samples at 4ºC in PBS-T	CG001672
4:00 PM	WET LAB	Thaw Pouch 4 at room temperature overnight Thaw Atera Plate 1 at 4ºC overnight	CG001672

Day 3: Instrument Run (9:00 AM–3:00 PM)

9:00 AM	WET LAB	Readiness test	CG001673
9:15 AM	WET LAB	Ethanol Drying	CG001673
9:30 AM	WET LAB	Loading and overview scan	CG001673
11:00 AM	HANDS-OFF	*Instrument run in progress*	CG001673
3:00 PM	WET LAB	Lab cleanup	-

The instrument run will take **~22 h** (but may finish sooner), followed by a **2.5 h** cleanup.

Two-Day Training (On Site)

Day 1: FFPE Tissue Preparation and Probe Hybridization (9:00 AM–4:00 PM)

9:00 AM	WET LAB	Training kickoff; incubate slides at 60°C for 2 h; program thermal cyclers	CG001671
9:30 AM	WET LAB	Prepare buffers and solutions for deparaffinization	CG001671
10:20 AM	WET LAB	Cassette assembly practice	CG001671
11:15 AM	WET LAB	Deparaffinization	CG001671
12:30 PM	WET LAB	Decrosslinking	CG001671
	HANDS-OFF	*Lunch*	
12:40 PM	WET LAB	Thaw probe hybridization reagents *(During thermal cycler step)*	CG001672
1:30 PM	WET LAB	Prepare Probe Hybridization Mix and start Probe Hybridization protocol on thermal cycler	CG001672
1:45 PM	HANDS-OFF	*Probe Hybridization in progress (16–24 h; incubate overnight)*	CG001672
2:00 PM	DISCUSSION	Review Day 2 schedule *Optional: Review fresh frozen protocol*	CG001672
2:30 PM	HANDS-OFF	Break (if necessary)	
3:30 PM	DISCUSSION	Instrument walkthrough	CG001673
4:00 PM	WET LAB	Thaw Pouch 4 at room temperature overnight Thaw Atera Plate 1 at 4°C overnight	CG001672

Day 2: Assay Workflow and Instrument Run (9:00 AM–5:00 PM)

9:00 AM	WET LAB	Thaw Post-Hybridization Wash Buffer Retrieve cassettes and start Post Hybridization Wash Thaw Ligation reagents, prepare Ligation Mix and start Ligation protocol on thermal cycler	CG001672
10:00 AM	HANDS-OFF	*Ligation protocol in progress* Obtain and reagents for amplification	CG001672
10:45 AM	WET LAB	Prepare Amplification Enhancement Mix and start Training Enhancement protocol on thermal cycler	CG001672
11:30 AM	HANDS-OFF	*Training Enhancement protocol in progress* *Lunch and Review Cell Segmentation and Instrument Operation*	CG001672
12:00 PM	WET LAB	Add Amplification Mix and start Amplification protocol on thermal cycler	CG001672
12:30 PM	HANDS-OFF	*Amplification protocol in progress* *Review stopping points*	CG001672
2:00 PM	WET LAB	Post Amplification Wash	CG001672
2:15 PM	WET LAB	Signal Boost	CG001672
2:35 PM	WET LAB	Readiness test	CG001673
2:45 PM	WET LAB	Ethanol Drying	CG001672
3:15 PM	WET LAB	Overview scan	CG001673
5:00 PM	HANDS-OFF	*Instrument run in progress*	CG001673

The instrument run will take **~22 h** (but may finish sooner), followed by a **2.5 h** cleanup.

Post-Training Closeout and Data Analysis

Users will receive a post-training closeout and data analysis call that covers:

- Data export and review of summary files
- Software onboarding
- Next steps for customer samples
- H&E *(optional)*

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Protocol Steps

Protocol Steps Overview

Process training slides according to:

- Deparaffinization and Decrosslinking steps in the Atera Gene Expression – FFPE Tissue Preparation Handbook (Document CG001671)
- All steps except Cell Segmentation in the Atera Gene Expression User Guide (Document CG001672).

Follow instructions in all reagent prep tables for two slides. Any additional guidance is specified below.

Sample Preparation

- Quality assessment of sections is not needed for the Atera Training Slides. However, quality assessment is recommended before running the Atera assay for best performance. The initial training call will provide information on quality assessment.
- Optimal sections for the Atera assay:
  - Have nuclei that are bright and punctate, as assessed by DAPI staining
  - Have no freezing or sectioning artifacts, as assessed by H&E staining
  - Have high-quality RNA (DV200 ≥30%) for FFPE samples
  - Have sections at recommended thicknesses (5 µm for FFPE, 10 µm for FF)
- Do not reposition sections after placement.

Step 1: Deparaffinization & Decrosslinking

- Remove Atera Training Slides from packaging and equilibrate at room temperature for **5–10 min**.
- After equilibration at room temperature, begin **2 h** incubation at **60°C** as described in the Atera Gene Expression – FFPE Tissue Preparation Handbook (Document CG001671).

Step 2: Probe Hybridization

- Follow probe hybridization mix preparation steps for an Atera Select panel, not an Atera WTA panel. Use the Atera Select Human Multi-Tissue Panel (A) to process training slides.

- Different pipetting techniques may be required depending on the protocol step. Consult protocol steps in the Atera Gene Expression User Guide (Document CG001672) for specific information.
- Avoid generating bubbles and do not pipette directly onto the tissue.
- When removing reagent, slightly tilt the adaptor and place the pipette tip at the bottom corner of the well.

Step 3: Post Hybridization Wash

No specific notes. Follow User Guide guidance.

Step 4: Ligation

No specific notes. Follow User Guide guidance.

Step 5: Amplification

- Ensure Amp Mix pipetting technique is followed due to reagent viscosity.
- The two-day training schedule has a **1 h** Training Enhancement step that is shorter than the Amplification Enhancement described in the Atera Gene Expression User Guide. The full Amplification Enhancement is used in the three-day training schedule.
- Do not perform the next step in the User Guide, Cell Segmentation, on Atera Training Slides.

Step 6: Ethanol Drying

- If performing the two-day training schedule, this ethanol drying immediately precedes instrument loading.
- If performing the three-day training schedule, this ethanol drying precedes a stopping point. Samples are stored at 4°C overnight.

Step 7: Instrument Loading

- If performing the three-day training schedule, perform the additional Ethanol Drying before instrument loading.
- Before loading, check cassettes for leaks and remove lids.
- Seat pouches flush in the reagent drawer and remove caps.
- Load plates in the correct orientation with barcodes facing toward the user.

Overview Scan and Field of View (FOV) Selection

- Select 16 contiguous FOVs on each cell pellet. As long as FOVs are contiguous, shape does not matter. For example, configurations such as 4×4 and 2×8 are acceptable.
- Avoid obvious defects such as debris, low cell density, and bubbles.

For information on starting an instrument run, consult the Atera Instrument Operation User Guide (Document CG001673).

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Expected Outputs

Expected Outputs Overview

Achieving the metrics in the table below indicates a successful training run. The shorter Training Enhancement may reduce sensitivity by up to 50% compared to the longer Amplification Enhancement in the three-day training schedule.

Metric	Target
Nuclear transcripts per 100 µm²	≥692
No warnings or errors	True
Percent of transcripts within cells	≥84%
Percent of empty cells	≤1%
Cells per 100 µm²	≥0.1