

Quick Reference Cards | CG001674 | Rev A

Atera Cassette

Assembly, Disassembly, and Use

For use with:

Atera Cassette Kit (2 cassettes) *PN-1001153*

Atera Instrument Accessory Kit *PN-1001179*

Atera

Document Revision Summary



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CG001674 | Rev A

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Rev A

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Atera

Cassette Components

Atera Cassette Components

The assembled Atera cassette contains multiple individual components that are used at various steps of the assay workflow.

This document contains detailed information regarding all parts of the assembled Atera cassette. For each part, key features and instructions regarding use during the Atera workflow are described.



Ensure familiarity with all components prior to performing an Atera experiment. Failure to properly handle the cassette and its accessory components may lead to compromised data or assay failure.

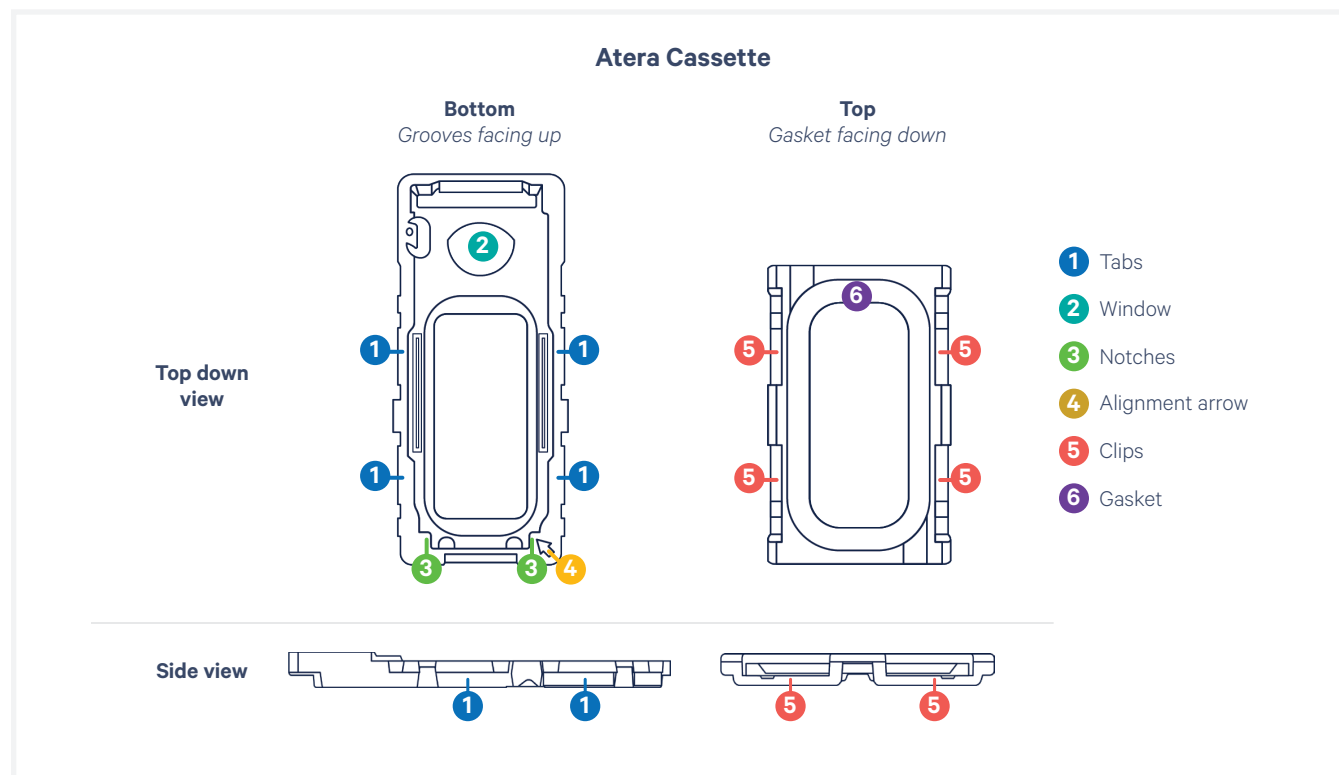
Atera Cassette Components



- | | |
|-------------------|-------------------|
| 1 Cassette bottom | 4 Cassette insert |
| 2 Tissue slide | 5 Cassette lid |
| 3 Cassette top | |

Atera Cassette

The Atera Cassette is used to create a well on the tissue slide for reagent addition and removal. The Atera Cassette is composed of a cassette bottom and cassette top. Key features of the cassettes are depicted in the figure below.



Cassette Bottom

- Tabs (x4): Engage with top cassette during assembly.
- Window: Circular cut out where label end of the tissue slide aligns.
- Notches (x2): Keeps the slide in place.
- Alignment arrow: Reference point at the bottom corner where the tissue slide should rest.

Cassette Top

- Clips (x4): Lock onto the cassette bottom tabs during assembly.
- Gasket: Creates a seal on the tissue slide.

Cassette top is symmetrical and can be assembled in any orientation.

Atera Cassette Assembly

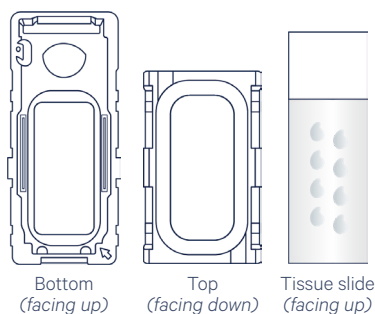


Before Assembly

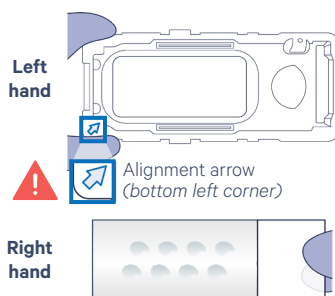
- Follow instructions carefully. Incorrect assembly can negatively impact assay performance.
- Assemble cassette on a clean work surface. Wipe the back of the slide before assembling cassette.
- 10x Genomics recommends using the Tissue Alignment Tool (PN-1001176) to ensure tissue is placed within the Sample Area. Tissue outside the Sample Area will not be analyzed and may be damaged during an instrument run.
- Tissue beneath the gasket will not affect cassette function. DO NOT attempt to remove it; doing so increases the risk of tissue detachment.

Cassette Assembly (perform on benchtop unless otherwise noted)

- 1** Place top and bottom cassettes on the bench, with top cassette facing down and bottom cassette facing upward.

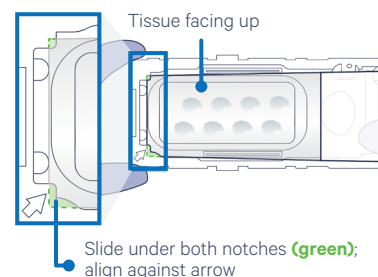


- 2** Rotate bottom cassette 90° clockwise. Hold bottom cassette (left hand; off the bench) and hold tissue slide by label end (right hand; with tissue facing up).

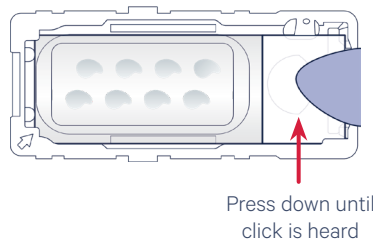


Failure to use the alignment arrow can cause slide misalignment. See page 6 for example images.

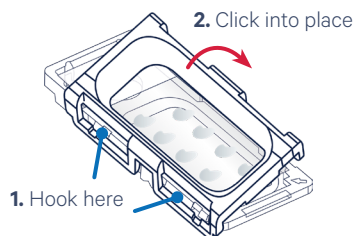
- 3** Insert bottom edge of slide under notches. Ensure long edge of slide rests firmly against the side of cassette marked by the alignment arrow.



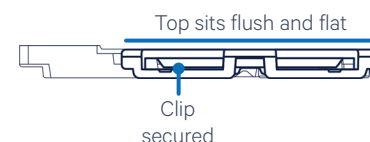
- 4** Press top of slide (label end) until it clicks into cassette. An audible click can be heard.



- 5** Place bottom cassette on a clean, flat surface. Hook the clips on one side of the top cassette to the corresponding tabs on the bottom cassette. Bring opposite side down and press down until clicks are heard and all four clips are fully secure.



- 6** Verify that all clips are completely secured over the tabs and the top cassette sits flush and flat to the bottom cassette. If not, apply pressure to secure any clips that are not fully engaged.



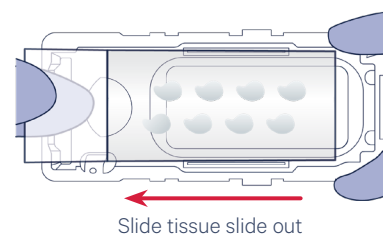
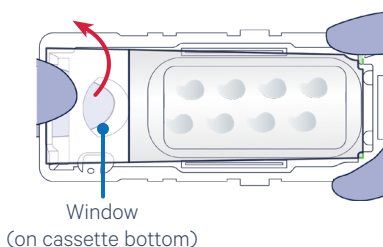
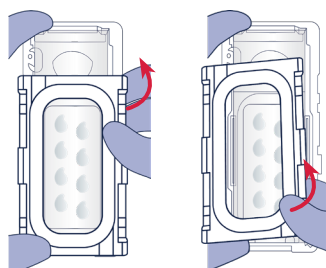
Incomplete engagement of clips can result in cassette leaking. See page 6 for example images.

Atera Cassette

Disassembly & Troubleshooting

Cassette Disassembly

- 1 While holding the cassette with one hand, disengage the top cassette clips with the other hand one at a time. Slowly remove top cassette.
- 2 Place finger on the underside of the bottom cassette (in the window). Apply gentle pressure upward until slide is released from the bottom cassette.
- 3 Gently move slide out from under the notches, being careful not to break or crack the tissue slide.



Examples of Assembly Failures

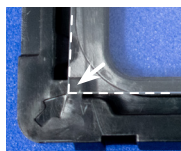
Slide Assembly Failure

Inspect slide alignment at alignment arrow.
Remove and reinsert slide to correct.

✗ INCORRECT



Gap at alignment arrow



Slide is outside the notch

✓ CORRECT



Slide is inside the notch and no visible gap at the alignment arrow

Cassette Assembly Failure

Inspect all sides for gaps.
Push down to correct.

✗ INCORRECT



Gap between top and bottom cassette (red).
Not fully clipped.

✓ CORRECT



Top and bottom cassette are flush.
No visible gap.

Atera Cassette Insert

Application, Removal & Use

The Atera Cassette Insert is used during specific steps to assist with reagent distribution. Key parts of the cassette insert are depicted in the figure on the right.

- Injection port: Adding liquid to insert.
- Extraction port: Removing liquid from insert.
- Notches: One top and two bottom; slides underneath the gasket of the top cassette, keeping it in place during use.

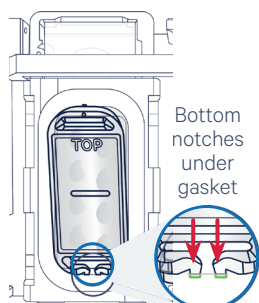


Cassette inserts are individually packaged in mylar bags. Once opened, inserts must be used within 30 min. DO NOT open the insert packaging until indicated to do so. Avoid touching the underside of the cassette insert.



Insert Application (perform on adaptor in flat and locked orientation)*

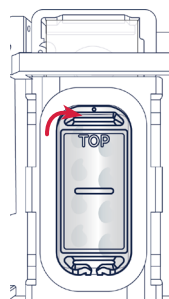
- 1** Slide bottom notches of insert underneath cassette gasket.



- 2** **Slowly** lower insert until top notch is underneath the cassette gasket and press down.



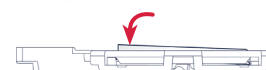
Remove and reapply if a bubble forms.



- 3** Visually inspect at eye level that insert sits completely flat (all notches are underneath gasket).



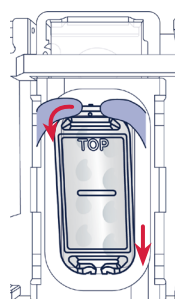
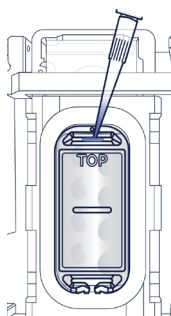
If insert is not flat, apply gentle pressure at the top of the insert and visually confirm insert is flat.



*If desired, forceps may be used to handle inserts.

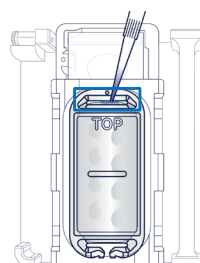
Insert Removal (perform on adaptor in flat and locked orientation)

- 1** Add 200 μ l PBS-T into the injection port.
- 2** Push the top of the insert toward the bottom of the gasket, then lift up to release.



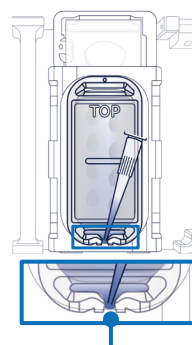
Using the Insert (perform on adaptor in angled orientation)

Reagent addition



Injection port
Add reagent here

Reagent removal



Extraction port
Remove reagent here



Position the pipette tip toward gasket. Do not touch the insert.

Atera Cassette Lid

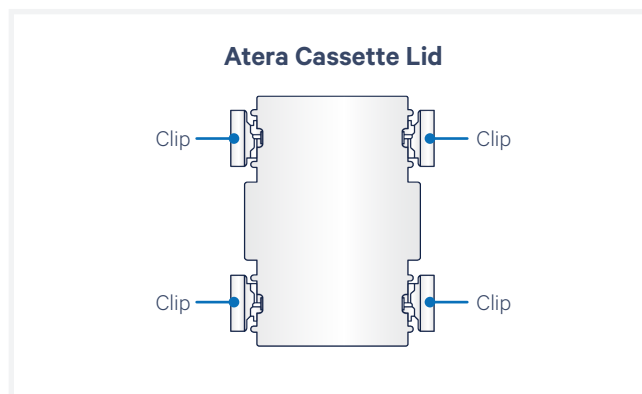
Application & Removal

The Atera Cassette Lid is used to seal the cassette during incubation steps. Only apply and remove the lid when indicated in the protocol. Key parts of the cassette lid are depicted in the figure below.

Instructions for application and removal are the same whether or not the Atera Cassette Insert is in use.

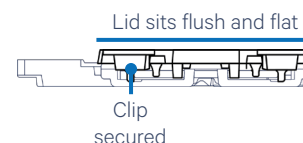
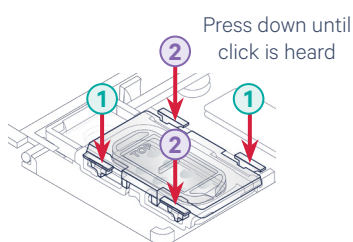
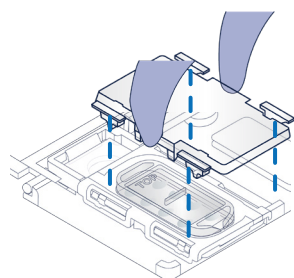
- **Clip:** Four clips are used to secure the lid to the cassette during application.

Cassette lid is symmetrical and can be assembled in any orientation.

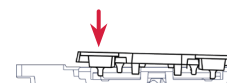


Lid Application *(perform on adaptor in flat and unlocked orientation)*

- 1 While holding the lid in the middle, align lid clips to the clips of the cassette top.
- 2 Press on two clips **diagonal** from each other until the clips click shut (1). Repeat with remaining two clips (2).
- 3 Visually inspect **at eye level on adaptor**. Clips **must be** completely secured over tabs with no gaps.

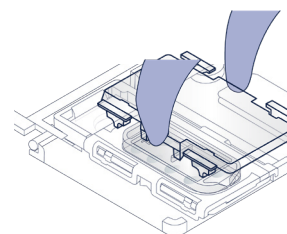
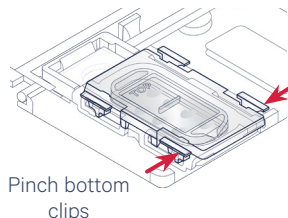
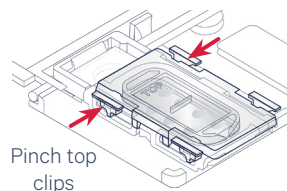


💡 If lid is not flat, push down on clips not fully seated and visually confirm lid is flat.



Lid Removal *(perform on adaptor in flat and unlocked orientation)**

- 1 Gently pinch the top two clips to unhook the lid from the cassette top.
- 2 Repeat for the bottom clips. This will release the lid completely.
- 3 While holding the lid in the middle, slowly remove the lid.

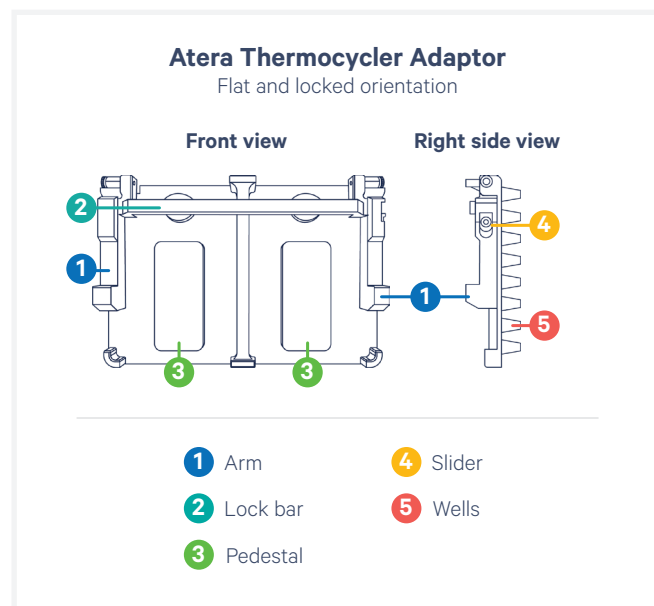


**Shown with insert. Steps are the same when performed with or without insert.*

Atera Thermocycler Adaptor

Orientations & Use

The Thermocycler Adaptor secures the Atera Cassettes onto the thermal cycler. Key parts of the thermocycler adaptor are depicted in the figure below.



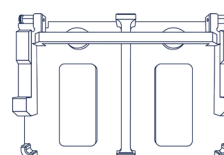
- **Arm:** Two arms on the right and left sides of the adaptor aid in angled orientation.
- **Lock bar:** Keeps cassette in place when in flat and locked orientation.
- **Pedestal:** Raised area where the cassette sits; ensures uniform heat transfer.
- **Slider:** Mechanism used to change orientations.
- **Wells:** Used to fit in tested thermal cyclers.

Thermocycler Adaptor Orientations

The Atera Thermocycler Adaptor is designed to sit in various orientations.

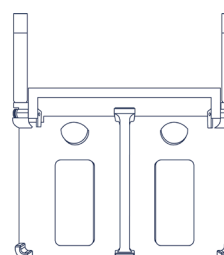
When prompted, users are instructed to perform specific assay protocol steps a particular orientation.

Atera Thermocycler Adaptor Orientations



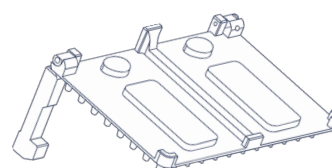
Flat and locked

Lock bar secures the cassette(s) in place.



Flat and unlocked

Lock bar and arms are open to allow for inserting or removing cassette(s) from thermocycler adaptor.



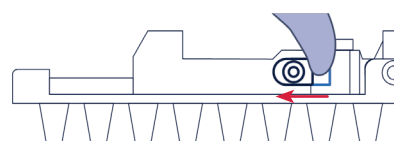
Angled

Arms hold adaptor securely in angled orientation.

Changing Adaptor Orientations

Pushing the slider down will allow the arms to swing freely to change orientations. Always hold the adaptor with the other hand while rotating the arms.

Unlocking Thermocycler Adaptor



Push slider down to unlock arms

Atera Cassette

Reagent Addition & Removal *(Without Insert)*

Reagent addition and removal methods (without cassette insert) differ depending on step in the Atera assay workflow. The two possible techniques are described below including their specific use cases. Always consult CG001672 Atera Gene Expression User Guide for execution details.

It is recommended that all pipetting steps are performed on the thermocycler adaptor in the flat and locked orientation.

General Pipetting Technique *(perform on adaptor in flat and locked orientation)*

General pipetting techniques apply to all steps except for when adding the Atera Amp Mix.

To add reagent to sample (in cassette without insert), pipette liquid in a U-shaped pattern around the sample.

To remove reagent from the cassette, tilt the adaptor with cassette to allow the liquid to pool in the bottom corner. Pipette liquid from corner to remove. Avoid touching the sample.

Pipetting Amp Mix Technique *(perform on adaptor in flat and locked orientation)*

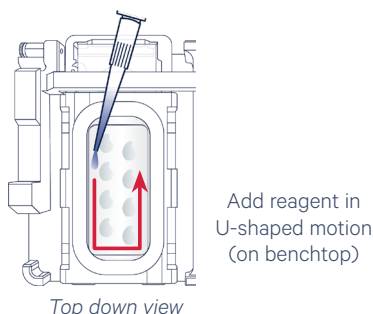
When adding the Atera Amp Mix, pipette directly over the sample quickly and evenly.

DO NOT pipette Atera Amp Mix along the side of the cassette.

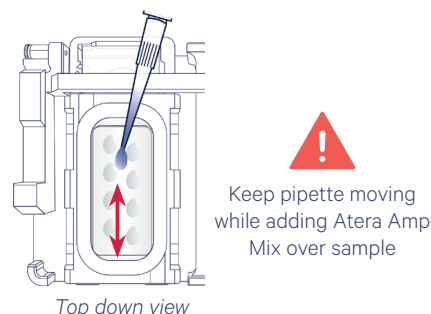
This is the only step in the protocol where pipetting over the sample is required.

It is critical that the pipetting motion is followed to maintain data integrity.

Adding Reagent to Atera Cassette



Adding Atera Amp Mix to Sample



Removing Reagent from Atera Cassette

